A Skin Substitute Reepithelialization Calculator for Natural, Synthetic, and Composite Skin Cell Scaffolds

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Abstract

Tissue engineering is a rapidly advancing field. Researchers around the globe have been working on novel ways to replicate the natural properties of tissues and organs. While new technologies enable increasingly complex therapeutics few of them have been found to be acceptable for clinical use. The skin is an excellent test bed for the techniques necessary for organ replacement. Skin is naturally regenerative, easily accessible and not as metabolically active as many other organs. This study analyzes published work to compare the epithelial wound repair rates of natural, synthetic, and hybrid tissue engineered skin substitutes to develop a wound repair calculator. Over 1,000 data points were utilized from 232 papers, linear regression produced a wound repair rate equal to the amount of wound repair per hour for each therapeutic class. Repair rates varied widely both within and across different therapeutics. Precision tests of the calculator's accuracy using 30 papers produced little concordance. The data reported in the tissue engineering papers examined here vary too greatly for accurate predictions of reepithelialization suggesting that the driving force in wound repair is not being effectively accounted for within the published works.

Dedication

This work is dedicated to all of the teachers who have encouraged me throughout my education. And to my family for their love and support, I will always be indebted to you for the gifts you have given me.

Acknowledgments

First I would like to express my deepest thanks to Dr. Sujata Bhatia, she is the true founder of this enterprise. She helped me discover my interest in tissue engineering, taught me its fundamental principles, and most importantly, has been unwavering in her encouragement. In a field of many amazing people your compassion sets you apart.

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Chapter I

Introduction

During the past hundred years the principal challenges facing modern medicine have shifted. The primary cause of mortality is no longer infectious diseases, instead degenerative and chronic conditions such as cancer and diabetes have become the greater unmet need (Armstrong, Conn, & Pinner, 1999). These maladies have specific molecular underpinnings which expand to impair organ and system function. Many current medical therapies, which broadly modulate the body's physiology, are ill suited to the precision applications necessary to truly cure these chronic conditions. For example, the current standard of care for a skin abscess in a diabetic patient is surgical drainage and debridement. However this procedure only mitigates further damage rather than actually improving the movement of nutrients in the extremity (Cahn & Y, 2014). An alternative strategy for treating such ailments is to circumvent the innate repair process by replacing the tissue with a synthetic analog. The need for engineered tissues extends beyond the realm of therapeutics into whole organ replacement. Demand for organ transplants already far outpaces the pool of donors by tens of thousands, and the projected need is accelerating ("Introduction," 2015).

The Potential of Tissue Engineering

Tissue engineering is a burgeoning field dedicated to using synthetic processes to supplement natural tissues. With advances in chemical engineering, cell culture and molecular biology it is now possible to replicate natural structures at nanometer resolutions. These therapeutics are called biomaterials, they are defined as products which can integrate with host tissue with minimal rejection. Such therapies are often comprised of three portions: replacement cells, cell scaffolds, and signaling molecules to promote proper function. Of these three components the scaffold is particularly important as it serves multiple roles in tissue regulation; scaffolds simultaneously influence the cellular adhesion, homeostasis and tissue ultrastructure. Tissue engineering products have already been applied clinically to replace a variety of different tissues (Williams & Bhatia, 2014). While the advancements in preclinical applications have been impressive there have been few products which actually graduate to clinical applications. There are many factors which contribute to this clinical barrier including an imperfect model of organ synthesis and repair, challenges in vascularization and the extensive testing necessary for validating novel therapeutics.

Ironically, the greatest impediment to clinical tissue engineering may be the success of laboratory tissue engineering. Researchers have combined different technologies to produce a plethora of potential therapeutics. Cell scaffolds, for example, can vary by substrate, polymer length, purity, pore size, ultrastructure, and fabrication

method. A single variable can elicit different cellular responses from an otherwise identical applications (O'Brien, Harley, Yannas, & Gibson, 2005).

Additional variables such as effectors, the type of cell being applied, cellular passage number, purity of the applied cells, and therapeutic application further conflates the issue. While the complexity of tissue engineering will likely necessitate a toolbox of techniques rather than a single solution, the plethora of variables seen in current studies dissuade standardization. This in turn prevents the formation of a foundation upon which further work can be based. Recent publications from the journal Tissue Engineering Part A utilized disparate cellular scaffolds to create full thickness epidermal tissues including polyvinyl alcohol-collagen-glycosaminoglycan (PVA)-(GAG) (Roessner et al. 2011), fibrin (Helmedag et al., 2014) and hyaluronic acid (Wu et al., 2014). Each of these designs had different beneficial properties but the absence of standard metrics and data analysis creates three different therapies for three different injuries rather than three methods of approaching one therapy.

The need for better standards in tissue engineering has been voiced by organizations such as The American Society of Testing and Materials (ASTM) and the Alliance for Regenerative Medicine, yet best practice guidelines are still lacking (Simon et al., 2015). The standards that do exist are only used sparingly. The National Institute of Science and Technology reference scaffolds, for example, are likely cost prohibitive for most research at $250 per sample. Similarly the ASTM tissue engineering guides are only suggestive outlines, rather than methodical practices. It is estimated that they are only used by around 0.3% of tissue engineering studies (Simon et al., 2015). Implementing universal standards is almost as complex a task as designing a therapeutic device, such

standards would need to bridge gaps between a wide variety of technologies while covering an equally wide variety of metrics. An ideal standard would produce precise measurements of cell seeding, adhesion, mortality, biocompatibility, distribution and proliferation across a wide variety of tissue engineering applications. Clearly, a set of standards rather than a single all purpose will be required to be applied to different therapeutics similarly to how different therapies will be required for different medical problems.

While standards would improve future work they cannot be applied retroactively to the thousands of studies already published. There may be unseen unifying trends already present in the hundreds of published results. Large scale analysis of this body of work could be used to find patterns in the absence unifying metrics. For my thesis project I would like to develop a cellular scaffold calculator to predict cell survival within skin scaffolds based on pore size, biomaterial concentration and cellular seeding density. Studies will be restricted to those containing hydrogel in situ applications of collagen, polyester and collagen-polyester composites scaffolds in rodents as skin tissue engineered therapeutics. These three different scaffold types are often used and serve as representatives of natural, synthetic and composite cell scaffolds, respectively.

By comparing between control and experimental groups the average effect of each component within the scaffold can be assessed. The large data set decouples the effect of the biomaterial from the effectors resulting in a quantitative measurement and allowing for relative comparisons across different scaffolds. This standardization will aid in the precision design of future cellular scaffolds, by relating properties of the cell scaffolds with predictable outcomes.

Tissue engineering therapies need to be designed to specifically mimic the tissue to which they are being applied. Experimental variables conflate the effects of the therapeutics design with specific application. The cell survival calculator will decouple these two factors by defining the expected results for specific designs *a priori*. For example, vascular endothelial growth factor (VEGF), is often used to improve the angiogenesis in tissue engineering therapeutics, but it also activates cell survival pathways (Byrne, Bouchier-Hayes, & Harmey, 2005). A single study that demonstrated improved cell survival when cells were applied with VEGF would be unable to identify if the increased survival would be due to the survival signaling or from changes in the surrounding tissue. The cell survival calculator would provide an effective baseline; a researcher could compare their study to the expected survival rates as well as compare their study to other studies run with VEGF.

Hitherto most meta-analysis of cell scaffolds has focused on physical properties and therapeutic efficacy while ignoring cell survival (Deng et al., 2015). Cell survival and engraftment are probably the most important metrics that can be attributed to therapeutics, as they represent how closely the microenvironment promotes homeostasis. The cell survival calculator could be used to start reframing the methodology used in tissue engineering. Ideally, it would help researches shift their focus from experimental testing novel products to hypothesis testing of predicted outcomes.

Natural Properties of the Extracellular Matrix

The extracellular matrix (ECM) is the network compounds that bind, organize and regulate cellular structure within tissues. Though chiefly composed of proteins, the makeup of the ECM is tissue and location dependant, cells extrude ECM proteins as they expand mitotically in response to the extracellular environment. It should be noted that conventionally an ECM refers to a naturally occurring organic connective tissue matrix, whereas a cell scaffold is a man made tissue engineering construct. The crosstalk between the ECM and the cells which it envelops influence the homeostasis and metabolic processes for the duration of the tissue's life (B. P. Chan and Leong 2008). Tissue function can be further regulated intrinsically through the composition, rigidity, topography and ultrastructure or extrinsically through signaling models like growth factors, cytokines and chemokines (Curtis, Dalby, & Gadegaard, 2006). The intrinsic and extrinsic signals influence differentiation, proliferation, localization and homeostasis (see figure 1).

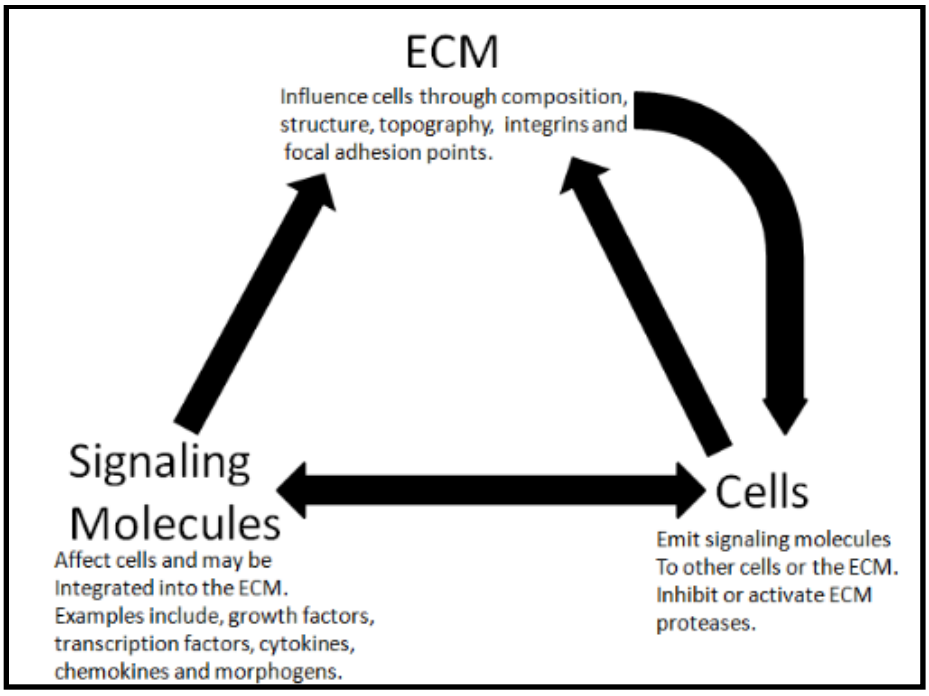

Figure 1. Relationships between the ECM, cells and signaling molecules. This visualizes the crosstalk between the three principle components of most tissue engineering therapeutics.

The ECM is mediated both through cellular extrusions and through interstitial proteases and inhibitors, this relationship creates a system of dynamic reciprocity (Birkedal-Hansen, 1995). These mechanisms help direct tissue response as a whole.

The ECM is mostly composed of collagen, in fact collagen is the most abundant protein in the human body. Collagen polymers naturally intertwine to form long fibrils. The tendons and cartilage have a high content of collagen fibrils; the elasticity and tensile strength that they require are representative of the properties which collagen can produce (Hynes, 2012). Additional polymers such as glycoproteins and proteoglycans are present throughout the collagen foundation of the ECM producing properties specific to each tissue (Buck & Horwitz, 1987).

Glycoproteins connect the ECM polymers or cells. Fibronectin binds, cells, collagen, heparin sulfate and fibrin, its motifs were used to develop the RGD adhesion peptides often used in cell scaffolding and cell culture (Järveläinen, Sainio, Koulu, Wight, & Penttinen, 2009). Elastin is a glycoprotein that allows materials to stretch, making them simultaneously plaint and resilient, it is found in tissues such as the lungs and skin (Bosman & Stamenkovic, 2003). Proteoglycans are polymerized disaccharides with covalently bound core protein, in addition to structural diversity properties are modified through bound co-receptors, morphogens, and cell adhesion proteins.

Signaling factors, such as VEGF or fibroblast growth factor (FGF) may be bound to specific ECM polymers (Zhu, Oganesian, Keene, & Sandell, 1999). These signaling factors may interact directly with cells or complex with other domains such as integrins to induce further signaling. The spatial relationships of receptor complexes influence the downstream signal cascades, introducing another layer of regulation (Wijelath et al., 2006). Free floating signaling molecules such as transforming growth factor-beta (TGF-B) may be bound by the ECM, they are released upon remodeling or cleavage by matrix metalloproteinases (Hynes, 2012). The ECM can also enable activation of TGF-B by binding inhibitors or presenting active sites to cellular receptors (Wipff & Hinz, 2008).

Adhesion molecules such as integrins and cadherins are built into the matrix, facilitating the localized attachment of specific cells (Uriel et al., 2009). Cellular focal adhesions connect receptors in the ECM to the cellular membrane and cytoskeleton, actively binding and signaling the cell simultaneously. The rate of ECM turnover is also governed by the concentration of cleavage sites within the polymers. Cellular secreted proteases break down nearby ECM components creating space for newly synthesized

networks. Tissue engineers are just beginning to imbue cell scaffolds with the complex spatial and temporal signals seen in natural ECMs (Ghosh and Ingber 2007).

The most important property of the ECM, and the most challenging to replicate synthetically, is the constant state of remodeling. Cells anabolically extrude matrix proteins and catabolically extrude proteases, building and deconstructing the local ECM as necessary (Larsen, Artym, Green, & Yamada, 2006). Building up the ECM at a rate greater than it can be broken down produces fibrotic tissue which in turn produces inflammation (Raghow, 1994). Conversely limited ECM extrusion weakens tissue cohesion and induces cell death through anoikis (Gilmore, 2005) . An artificial cell scaffold must be either bioabsorbable with a degradation rate equal to ECM synthesis or be maximally biocompatible, producing a minimized inflammatory response. The remodeling rate of the ECM varies by tissue and metabolic state. It is exceedingly challenging to produce a device which can meet the mechanical and temporal requirements of the natural ECM.

The cell scaffold survival calculator will inform future studies by providing benchmarks for both prescriptive design and post hoc analysis. Prescriptively, the cell scaffold survival calculator can be used to ensure that the therapeutics' design and the clinical goals are aligned. If cells are not projected to survive the lifespan of the therapeutic, redesigns may be necessary for maximal efficacy. Conversely when a therapeutics cell survival is different than what is predicted it may indicate that the scaffold contains elements which significantly influence survival. These discrepancies will drive tissue engineering research by highlighting design elements to avoid or incorporate for future work.

Discrepancies Between Cell Scaffolds and the Natural ECM

Tissue engineering products can be composed of natural biomaterials, which are organic compounds produced in nature, or they can be composed of synthetic biomaterials which are man-made. Cell scaffolds are designed to be biometric but there is a wide gulf between what can be fabricated and the properties cells experience within tissues. Natural biomaterials often exhibit emergent properties, producing characteristics greater than the sum of their parts. Mollusk shells for example, are composed of aragonite, but the complex patterning makes the shell 3,000-10,000 times tougher than inorganic aragonite (Dean, Swanson, and Summers 2009).

Table 1
Physical properties of differently sourced elastin.

The synthetic elastin described here is a recombinant human tropoelastin, cross-linked with bis(sulfosuccinimidyl) substrate.

Protein	Origin	Elastic modulus	Strength (stress at break)	Extensibility (strain at break)
Elastin	Bovine nuchal ligament	1.1 MPa	2 MPa	150%
	Porcine aorta	0.81 MPa	1.02 MPa	103%
	Synthetic	0.22–0.28 MPa	~ 0.4 MPa	200–370%

Note: Table originally reported in (Muiznieks and Keeley 2013).

Current fabrication methods are unable to reproduce the complex patterns seen in nature, so even cellular scaffolds made from identical macromolecules have different physical properties than the ECM observed in tissue. This discrepancy is present at the microscopic level and then expands at the level of tissue wide ultrastructure (see Table 1).

One strategy use to minimize this discrepancy is to use blends of natural and synthetic biomaterials, called hybrid or composite scaffolds. These scaffolds afford the tunability of synthetic scaffolds and the biocompatibility of natural scaffolds.

As the properties that go into making the ECM influence the properties of the tissue, the fabrication parameters affect the properties of the cell scaffold. Collagen cell scaffolds are affected by the material source, the pH at polymerization, temperature, ionic strength and polymer concentration, macromolecule purity and the presence of any adulterants (Dado & Levenberg, 2009). These characteristics impact the mechanical properties, structure and porosity. While these factors have been shown to be important they are not always reported in papers, this is partially due to the technical challenges involved in proper evaluation. Structural measurements are particularly challenging as there are currently no automated image analysis methods.

In addition to the development of novel therapeutics the cell scaffold calculator could be used to discover unknown biological mechanisms. Comparing interactions across multiple applications could identify effectors which produce a significant change which is only apparent in aggregation rather than single studies. These unknown factors may represent undiscovered metabolic mechanisms and could be fruitful avenues of

research for future studies. Alternatively these effectors could be used to interrogate cellular repair and growth in model systems.

Tissue Engineering

Cell scaffolds are often classified by three categories; material, fabrication, and assembly. These three properties have the greatest effect on the overall relationship between the scaffold and the host tissue, but they are by no means an exhaustive accounting of all the factors which produce a physiological response. The materials are either naturally compounds, synthetic compounds or a composite of the two. Natural materials integrate well with the body's homeostasis but are often harder to manipulate. Tissue engineers have greater control of synthetic products, but synthetics are more likely to produce a response from the body through processes like inflammation (Jiang, Liang, & Noble, 2010). Fabrication can be classified as either top down or bottom up. In top down fabrication complex structures are disassembled to isolate the necessary therapeutic, such as when tissues are decellularized to obtain an ECM which is then reseeded with cells to create tissues (Bechtel et al., 2003). Bottom up fabrication takes place when component macromolecules are assembled to produce a novel structure, a hydrogel which is made with cross-linking polymers once in place is an exemplary product. Engineered products are either assembled ex vivo or in situ. Ex vivo therapies are processed in the lab and then transplanted into the patient, in situ therapies are applied directly to the patient, assembly takes place at the therapeutic site ("Wiley," n.d.). All of

these different factors must be considered together to understand the properties of the scaffold and tissue product.

Advancements in tissue engineering have afforded researchers the ability to replicate some of the properties of natural tissues but no scaffold has successfully recapitulated all of the natural complexities seen in the ECM. In trying to satisfy all of these distinct needs tissue engineering therapies are becoming increasingly complex. Functionalization and fit for purpose design are often used to tailor the therapeutic to the needs of the individual disease (Haag, Jungebluth, and Macchiarini 2013). Unfortunately this complexity increases the amount of splintering within the literature. Tissue engineering therapies applied to identical diseases may have completely different properties. Differentiated human embryonic stem cells and polytetrafluoroethylene patches can both be applied to a myocardial infarction but they produce vastly different physiological responses (Lakshmanan, Krishnan, & Sethuraman, 2012) (Shiba et al., 2012). To effectively draw comparisons across different kinds of scaffolds in this thesis it is important to impose a set of selection criteria.

The positive selection is described in detail in Chapter II: Selection Criteria, but with the variety of cell scaffolds to chose from, the negative selection criteria is just as crucial. The scaffolds analyzed in this thesis were chosen to be representative of biomaterials often used in tissue engineering. Scaffolds with unique physical and biological properties were excluded from analysis. Polyhedral oligomeric silsesquioxane poly(carbonate-urea) urethane for example, is a highly biomimetic compound with promising tissue engineering applications (Williams & Bhatia, 2014). But it is a synthetic, non degradable, inert compound which is not reflective of natural compounds

or most synthetic biomaterials. Biomaterials which were rarely used in animal models were also excluded as they would not provide enough data for comparison. Similarly, highly functionalized scaffolds or scaffolds with many component monomers were excluded as it would be difficult to discern the effect of each biomaterial in the presence of numerous other bioactive motifs.

Tissue Engineering Challenges

Despite advanced functionalizations cell scaffolds are intrinsically different than a natural ECM. In an organism the ECM is extruded during gestational development, the ultrastructure expands concurrently with cellular mitosis. After the tissue has grown to its maximal size the ECM will then undergo physiological changes with the surround tissue (Rozario & DeSimone, 2010). By contrast cellular scaffolds are assembled first and then exposed to cells. An effective scaffold must concurrently allow for three discrete goals. First cell scaffolds must bind exogenous or host cells, second it must allow, if not facilitate, engraftment into the host tissue. Third, the cell scaffold must create an environment conducive to the restoration of normal tissue function. As the ECM is produced during tissue development the first and second goals are not seen simultaneously in nature. Though the ECM will replicate such properties sequentially during wound repair remodeling steps they are initiated over time and at small scales (Midwood, Williams, & Schwarzbauer, 2004). Materials that are amenable to fabrication

are often ill suited for structural stability and vice versa. Thus the ideal cell scaffolds should exhibit properties beyond the scope of any single ECM.

Scaffold Functionalization

To induce the desired properties within the therapeutic tissue engineers must be mindful of how cell scaffolds influence the overall tissue. The cell scaffold survival calculator will improve the practice of scaffold functionalization by defining the expected survival of cells grown within biomaterial matrices. Future studies will be able to identify the efficacy of each individual functionalization, developing a toolbox with which to tailor the overall tissue response.

Current products already contain specific signaling motifs to produce the intended effect. One of the first things to consider is how cells bind to the scaffold; this is determined by many factors such as porosity, the substrate and the presence of any adhesion sites. Porosity must be carefully regulated, if the space between cells is too large the tissue will not maintain cohesion, if there is too little space between cells they will not be able to migrate into the scaffolds core (Murphy, Haugh, & O'Brien, 2010). Integrated ligands or adhesion motifs can be built into scaffolds to recruit cells (Schussler et al., 2009).

The three dimensional structure of an ECM dictates its overall properties; similarly the structure of a scaffold dictates its efficacy and engraftment into the hosts

tissues. Vascularization limits the size of an engineered tissue. Oxygen will only diffuse across a distance of about 100 μm, in the absence of proper gas exchange a tissue will develop necrotic regions (Haraguchi et al., 2010). Successful therapeutics must allow for the growth of new vasculature and allow for integration with the host vasculature at a rate proportional to tissue growth. These two properties are called angiogenesis and anastomosis respectively. Growth factors such as VEGF can improve vascularization (Ghanaati et al., 2011; Kucera, Doss, Dunn, Clemson, & Zwischenberger, 2007). In spite of these functionalizations the maximal size of in vitro engineered tissues is a few millimeters thick (Kaully, Kaufman-Francis, Lesman, & Levenberg, 2009). This remains a significant limitation in the clinical utility of engineered tissues.

Appropriate loading of the cell scaffold, through design informed by the cell scaffold calculator will enable better products within the constraints of oxygen diffusion. The addition of more cells than the therapeutic can reasonably support only accelerates necrosis. The cell scaffold calculator can be used to suggest a maximal loading, the point at which higher concentrations of cells most likely die without conferring therapeutic benefit. More broadly the methodology employed in the cell scaffold survival calculator can be adapted to any quantitative variables.

Tissue engineered products must be either permanent, maintaining lifelong biocompatibility or temporary where they are replaced by the natural ECM. Permanent cell scaffolds are unencumbered by considerations of degradation rates but they produce a higher risk of acute inflammation (Anderson, Rodriguez, & Chang, 2008). Bioabsorbable biomaterials, which can be metabolically broken down within the body, allow for natural repair and integrate well with host tissues after the initial

implementation (Tamai et al., 2000) . However most bioabsorbable scaffolds are broken down by stochastic hydrolysis, ECM turnover is mediated primarily by secreted proteases, which is a more regulated process. While it is impossible to precisely replicate the regulated remodeling of the ECM with the chaotic degradation of cell scaffold polymers, hybrid biomaterials can be used to control remodeling. Laboratory measurements are often used to provide a rough estimate of remodeling rates to prevent catabolic tissue prolapse (Ike, Shimizu, Okada, Ikada, & Hitomi, 1991).

The cell scaffold survival calculator will inform the precision of predicting ECM remodeling. Understanding the expected population of living cells is the first variable necessary for building a mathematical framework for ECM extrusion rates. Eventually, remodeling will be derived algorithmically rather than empirically. The calculator could be used to produce guidelines for future biomaterials. Understanding the expected cell death over device engraftment can be used to define target survival rates for new cell scaffolds. Discrepancies between the expected and observed cell survival can be used to identify the best therapeutic strategy.

For example, an observation that 70% of cells do not survive past day 14 within most scaffolds could be used as a benchmark. It may be that scaffolds which have completely biodegraded by this point produce better host response. Conversely it could be that scaffolds which are still in place allow for host cells to take over the repair process from the exogenously introduced cells. The cell scaffold calculator could be used to compare between the two methodologies, identifying the best therapeutic strategy. ECM remodeling varies by tissue. The cell scaffold calculator will define expected results allowing for informed design and comparisons. Remodeling rates could be

compared across tissue rather than across therapeutics. A better understanding of cell scaffold remodeling would inform the broader physiological changes occurring during tissue repair. A tissue's regenerative capacity is determined by many factors such as inflammatory response, tissue injury and the abundance of host stem cells. Different organs have markedly varied abilities to repair, from the highly regenerative liver to the brain which has limited ability to repair. Despite the range in capabilities the overall repair process is often conserved across tissues and the ECM is a crucial mediator to the tissue's regenerative potential (see Table 2 in the appendix, page 52). Understanding the remodeling process will provide a more contiguous spectrum of classification for the tissue repair process. This could lead to improved therapeutic efficacy through application at the optimal phase of wound repair and a more detailed understanding of the requirements of regeneration. This could lead to new therapeutic targets using cell scaffolds as the primary biological effector.

Hydrogels

Hydrogels are malleable solids made up of matrices in an aqueous solution. They are amenable to skin tissue engineering as they can be shaped to fit the required portion of skin. This study focuses on papers published with hydrogel cell scaffolds for several reasons. First hydrogels are often utilized for dermal applications, providing both a range of previously published works and a built in application for future work. Second, utilizing hydrogels circumvents potential computational complexities. It would be challenging to

compare survival in scaffolds with radically different size, thicknesses and application areas. Cells within a hydrogel are embedded within the scaffold stochastically, as the cells already vary between applications, there will be no explicit differences in ultrastructure design.

Therapeutic cell scaffolds must mimic the ECM, remaining pliant enough to allow for movement while strong enough to provide structural support to multiple dermal layers and avoid tears. This study will focus on cell scaffolds used in skin therapies using collagen, polycaprolactone and hybrid collagen polycaprolactone scaffolds.

Skin Tissue Engineering

The skin is the largest organ in the body and is made up of three dermal layers. It simultaneously provides structural support and serves as a barrier to opportunistic pathogens. The skin is one of the most regenerative organs but all three layers can only be repaired over a small surface area (Lei, You, & Andreadis, 2013). The outermost layer is the epidermis, it is mainly made up of keratinocytes. The ECM forms a heparin sulfate basement membrane containing proteoglycans to connect the lower levels, the dermis and epidermis. The basement membrane signals keratinocytes to differentiate into dermal cells with growth factors and cytokines (Breitkreutz, Mirancea, & Nischt, 2009). The dermis has a collagen ECM as well as elastin fibrils embedded with proteoglycans to bind cells. Fibroblasts which secrete the ECM are the primary cell type found in the dermis though macrophages are also present to prevent infection (F. A. Auger, Lacroix, and

Germain 2009). The hypodermis connects the dermal layers to muscle or bones via an elastin network, and is chiefly made up of macrophages, blood vessels, fibroblasts and adipocytes. Skin replacements are broken into full and partial therapeutics depending if they include all three layers or merely the epidermal layer. Tissue engineered dermal layers must integrate into the host structure, be non-immunogenic, provide a temporary barrier and allow for self repair throughout the patient's life.

Over 20 tissue engineered skin products are currently on the market (F. A. Auger, Lacroix, and Germain 2009). These products utilize a wide range of strategies including acellular and cellular applications, utilizing xenograft byproducts, keratinocytes, and neonatal foreskin (Jones, Currie, & Martin, 2002; Kirsner et al., 2012; D. Q. A. Nguyen, Potokar, & Price, 2010; Purdue et al., 1997). Currently only the therapeutic Apligraf has received FDA approval to be used as a full thickness skin substitute. Apligraf utilizes fibroblasts, seeded within a type 1 collagen matrix with a layer of differentiated keratinocytes (S. Hu, Kirsner, Falanga, Phillips, & Eaglstein, 2006) . The tissue contains growth factors and cytokines and is a solid barrier against pathogens and physical perturbation. Interestingly DNA from Apligraf cells was not found past 4 weeks when applied to patients, yet the wound closure was improved when compared to controls. This may suggest that Apligraf's benefits are not conferred by cells (S. Hu et al., 2006).

Collagen

Collagen is the most abundant protein in the animal kingdom. It is often spoken of as a singular entity but collagen is actually a family of 20 proteins, all of which contain a repeating three amino acid motif of glycine, proline and a variable third amino acid. The variety of collagen proteins underscore its utility and importance but 80-90% of the collagen within the human body is comprised of types I, II and III. Hydrogen bonding between the glycine side chain and carbonyl group of the adjacent peptide allow collagen polymers to intertwine into triple helices. These helices then align into long fibrils which interconnect to produce the ECM see figure 2.

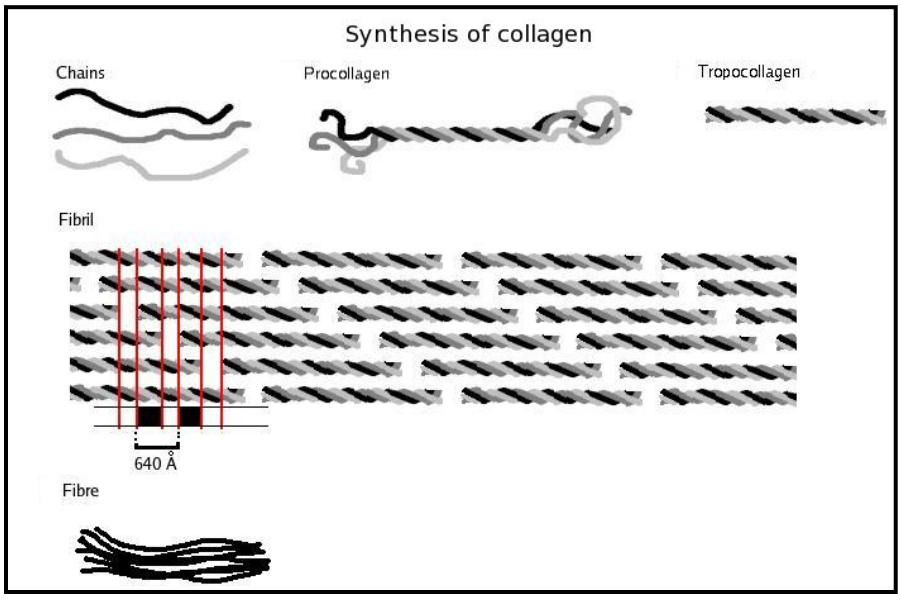

Figure 2. The Structure of Collagen Macromolecules. Collagen amino acid chains combine to a form triple helical procollagen structure. Cleavage of the N and C terminals produces the more water soluble Tropocollagen. Tropocollagen molecules are covalently bound to form fibrils which are the principal component of the ECM (fr.wikipedia, 2005).

Though extruded collagen proteins self assemble into fibrils, decellularized whole fibrils are non soluble. Collagen hydrogels often contain separate fibers which are then crosslinked once the hydrogel has been placed using temperature or pH gradients (Drury & Mooney, 2003). This study will focus on Type 1 collagen, specifically tropocollogen. Type 1 is the principal component of the skin and tropocollogen is more soluble than other collagen structures. Type I tropocollogen is the most used form of collagen hydrogel and also reflects the natural tissue.

Polyesters

Polyesters are polymers formed through condensation reactions between a carbonyl hydrogen of one molecule and a hydroxyl groups a second molecule. This creates an ester which links the two carbon chains, R-0-R. While polyesters have excellent biocompatibility and can be manipulated during synthesis to meet design specifications, their chief advantage is their biodegradability. Over time the esters will stochastically hydrolyze breaking the macromolecule down into component polymers. Most polyesters break down into units which are readily metabolized (Gunatillake & Adhikari, 2003). PLGA for example, hydrolyzes into lactic acid and glycolic acid which are readily metabolized by the body. Because the units can be easily interchanged

polyesters are tunable, the length and number of units can be chosen for the tissue of interest. The ratio of lactic acid to glycolic acid in PLGA can be modified to affect a scaffold's melting temperature, strength and degradation rate. Polyesters have already been used for FDA approved drug delivery devices. Capronor for example, is an implanted birth control sub dermal PCL device which can be used to deliver levonorgestrel to a patient for a year (Armani & Liu, 2000).

One drawback of polyesters is that they are relatively hydrophobic in a biological microenvironment. Scaffolds will often be functionalized with polar compounds such as ZnO to improve solubility. This contributed to the relative paucity of polyester hydrogels when compared to collagen hydrogels. This study was intended to focus exclusively on polycaprolactone scaffolds but the lack of suitable literature necessitated the broadening of the scaffolds utilized in this study. While this increased variability reduces the specificity of the findings it also broadens the application of the work making it more representative of the polyester family and scaffolds as a whole.

Chapter II

Research Methods

The methods for developing the cell survival calculator are outlined in this section. Data published in a previous works were used for modeling by regression analysis to identify the rate of wound repair. The accuracy of the reepithelialization calculator was assessed by comparing the difference between expected values and those published.

Selection Criteria

This study will be focused on hydrogels applied to murine skin therapeutics utilizing collagen, polyester or hybrid collagen polyester cell scaffolds. These specific tissue engineering products were chosen because they will provide enough data for statistical analysis, while maintaining properties similar enough to compare across studies. Hydrogels are preferable to other tissue engineering applications as they will not be dependent on spatial structure or design. Identical compounds at uniform concentrations should behave identically across different studies. Hydrogels make excellent skin therapeutics because they are easily applied, can satisfy the tissues

structural requirements and can cover the affected area without concern for immediate engraftment with neighboring tissue. Murine animal models are often used to test preclinical skin therapeutics, providing a sufficient range of studies to meet the selection criteria. Additionally once therapeutics are defined as full or partial, skin treatments are in many ways two dimensional. Therapeutics applied to another organ would have a many spatial discrepancies between different applications. Lastly, the three cell scaffold types were chosen to serve as representatives of natural, synthetic and composite cell scaffolds. Taken together these three scaffolds will represent the range of tissue engineering capabilities, from natural to synthetic compounds. These scaffolds are widely used and will provide enough citations to build an effective algorithm while being representative of the larger cell scaffold population.

Literature review and indexing

Previous published studies will provide data for the cell scaffold survival calculator. Studies will be limited to in vivo, hydrogel, cell scaffolds applied to rodents comprised of collagen, polyester or collagen-polyester composites scaffolds. Collagen and polyester are often used biomaterials and are suitable representatives of natural and synthetic scaffolds in general. Up to one hundred papers for each scaffold type will be utilized to provide a robust sample size for a training data set. Though all of the described metrics will not be included in the paper every effort will be made to select papers that

include quantitative measurements of all of the metrics described. Cell survival and infiltration information will be the most important metrics, papers which contain this data will be preferentially selected. Three scaffold types will help reduce bias, by comparing cell survival across different scaffolds it will be easier to determine baselines and thereby eliminate outliers. Relying on a single scaffold type could skew the data, overemphasizing a specific interaction between the biomaterial and the cells. Developing an index of the relationship between cell scaffold and biocompatibility in untreated scaffolds will represent the nominal survival rates. The material, fabrication method, porosity, concentration, cellular seeding density, cellular survival, cellular infiltration and the addition of any signaling molecules will be noted as reported quantitatively in each study. The purpose of the calculator put forward in this proposal is to predict the change in cellular survival for each unit change in these metrics.

Regression Analysis of Reepithelialization

Once indexed the metrics identified in part one of the methods will be utilized to develop statistical relationships between the scaffolds properties and cellular reepithelialization. Qualitative factors will be compared by ANOVA and T-tests while quantitative factors will be compared by regression analysis. Linear models will be tested first but logarithmic, quadratic and exponential will also be reported so that a higher degree polynomial function would be identified in the event of a better statistical

fit. Such multi order polynomials may not be amenable to the calculator being developed here but they would still provide information about the relationship of the metric with survival. The relationship between each metric and cell survival was visualized graphically and the statistical results were reported. Transformations and multiple models will be applied to identify the equation which best reflects the changes in cell survival. The coefficients of each of these models will be used to develop the calculator. Confidence intervals for these calculations will be set at 95% and the p value threshold will be set at 0.05.

ANOVA Analysis

Once the initial regression model has been generated an analysis of variants (ANOVA) will be performed to answer two questions. First, it will identify which metrics have a significant effect on reepithelialization. The interaction between two variables must produce a p value <0.05 to be considered significant. Multiple iterations may be necessary to produce the model which best reflects the changes in cellular survival. The adjusted R square value, which is a measure of the relationships between variables in a multiple regression analysis will be utilized to rank the different iterations. The coefficients produced in the model with the highest R square value will used for the final calculator. ANOVA analysis will identify if any of the indexed metrics have a significant effect between each other. This is particularly important because it will

identify any multicollinearity, that is to say multiple metrics which produce the same effect in survival. Multicollinearity is quite possible as many of these metrics are interrelated at a physical level but may not share a correlative relationship. For example a scaffolds concentration and porosity may both be the result of type of cross-linking and hence be physically correlative but they could produce two different cellular responses. While many scaffolds can serve as drug delivery vehicles non biologics will not be considered for this thesis. The predictive power of each metric will be reported, any multicollinearity will be identified.

Reepithelialization Accuracy Testing

Ten additional studies for each type of scaffold will be used as a validation testing the accuracy of predicting cell survival. The expected survival as predicted by the calculator will be compared to the survival observed in the course of the study. Discrepancies could indicate a need to reformat the model, alternative models which were passed over in the course of modeling will be tested as well to identify unaccounted factors which do not fit the model. Remaining discrepancies between the calculator and the observed results after running through all of the parameters will indicate that the survival is being affected by an unreported metric. If the system is accurate variables such as the biomaterial and seeding density could be used to predict the cellular survival in a wide range of therapies using these scaffold types.

Chapter III

Results

Data from 232 papers were successfully used to develop a cell scaffold calculator. Wound repair rates varied greatly both within and across classes of cell scaffolds. Expected results did not correlate with observed results reported with the test group of published papers. The major determinants of reepithelialization were not represented in this analysis.

Study Summary

Data from 232 papers were utilized in the generation of the cell scaffold calculator. As the papers often contained multiple scaffolds over the course of multiple time points this resulted in 1016 individual data points quantifying the reepithelialization of 444 cell scaffolds, as outlined in Table 3. Several journals contained more suitable studies than most other journals. Biomaterials, Burns, Tissue Engineering Part A, and Materials Science Engineering were often utilized, but a wide variety of journals were used as visualized in figures 3a-c. Journals which contributed only one paper were labeled as

other. The variety of journals and experiments limited the bias of any individual reporting system.

Table 3			
Breakdown of Data Included in This Analysis.			
	Number of Papers	Number of therapeutic tests	Number of data points
Natural Scaffolds (Collagen)	100	196	438
Synthetic Scaffolds (Polyesters)	83	158	363
Hybrid Scaffolds (Collagen-polyester hybrids)	49	90	215
Total	232	444	1016
Note: The number of therapeutic tests includes both hydrogels and controls without therapeutics. The number of data points is the sum of reepithelialization measurements reported for all scaffolds.			

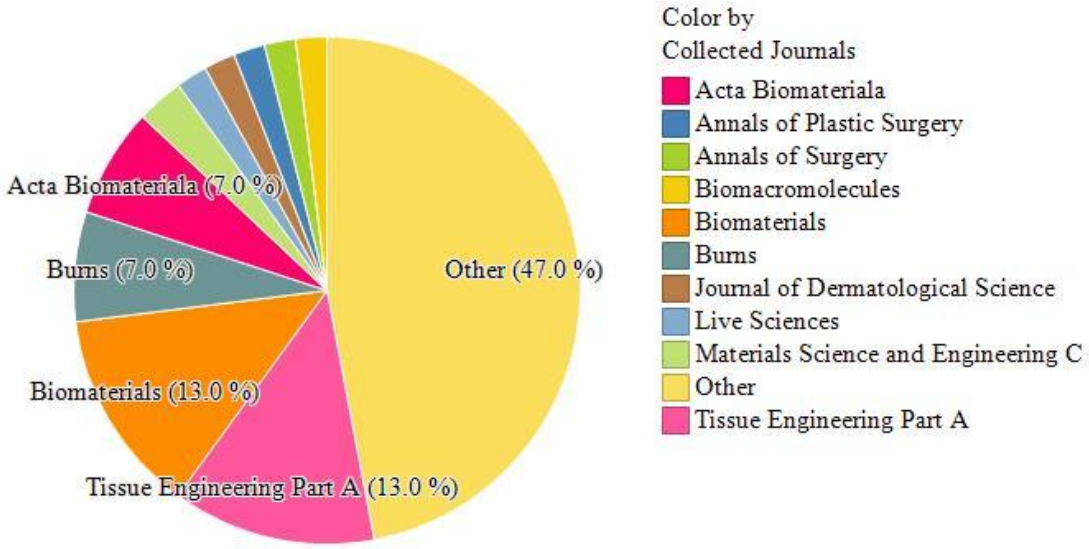

Figure 3a. Journals with Collagen Therapeutics Utilized in This Study. Of 100 papers reporting collagen therapeutics which were utilized in this study most were from Tissue Engineering Part A, Biomaterials and Burns.

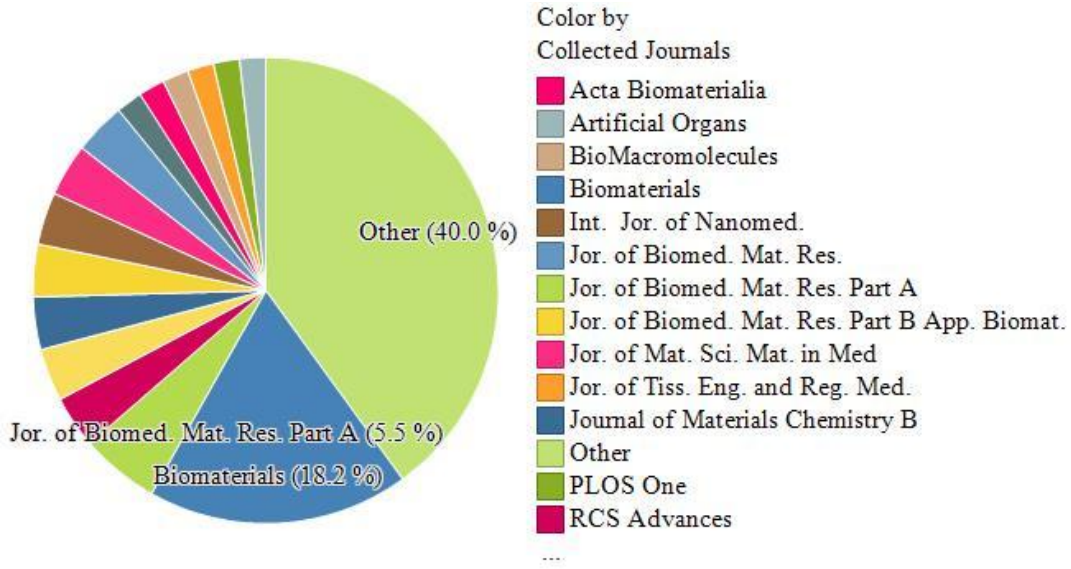

Figure 3b. Journals with Polyester Therapeutics Utilized in This Study. Of 83 papers reporting collagen therapeutics which were utilized in this study most were from Biomaterials, The Journal of Biomedical Materials Research Part A and Materials and RSC Advances.

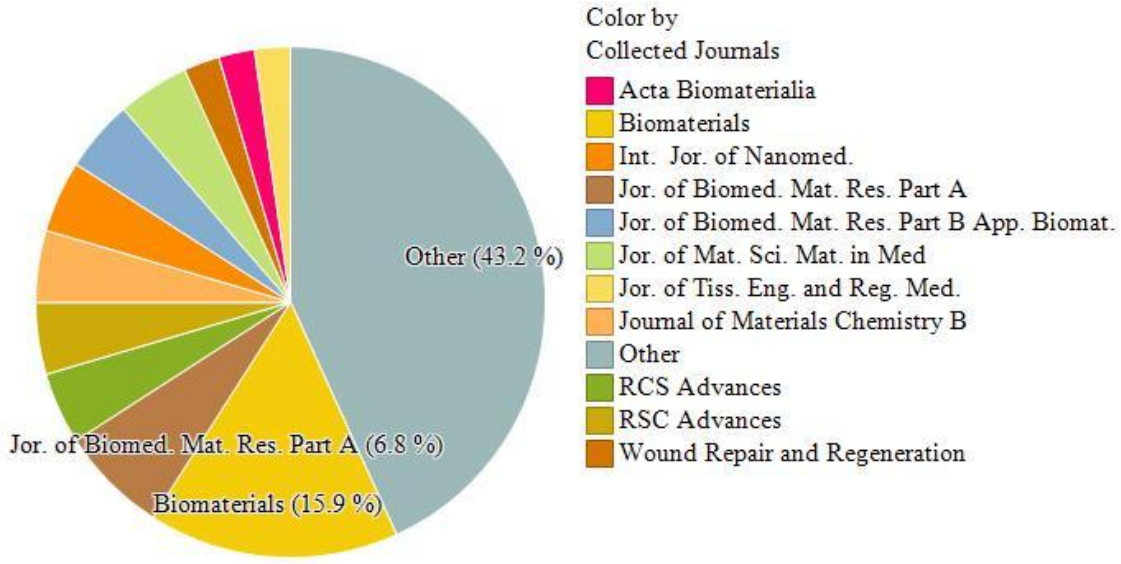

Figure 3c. Journals with Collagen Polyester Therapeutics Utilized in This Study. Of 49 papers reporting collagen therapeutics which were utilized in this study most were from Biomaterials, Tissue Engineering Part A and RCS Advances.

Though the information reported in journals varied, the most commonly used metric of survival was reepithelialization. This was quantified by the percentage change in area of the wound. For example a 4 cm^2 wound that was reduced to 2 cm^2 would be reported as 50% healed. While some studies made use of immunohistochemistry, GFP producing seeder cells or cell proliferation assays these were the uncommon and in total constituted <12% of the total number of studies (see figure 4). Where possible metrics were converted to % wound healed if possible.

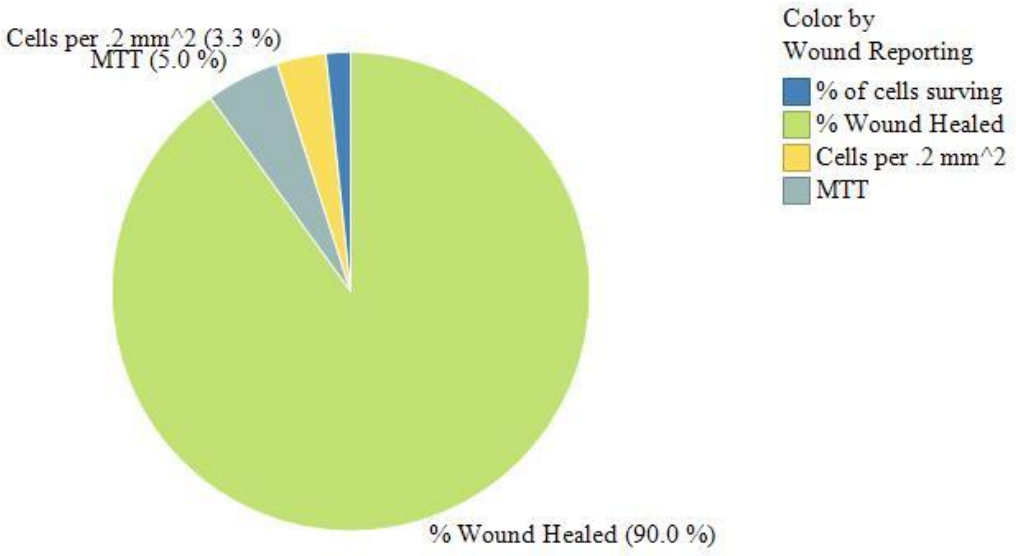

Figure 4. Cellular survival metrics reported across all 232 papers. Papers reported on cell survival utilizing a variety of metrics, percent wound healed was the most often used metric. When possible metrics were converted to percent would healed to standardize comparisons.

Though there were slight differences across the scaffold classes, approximately 2/3 of the studies utilized mice as the model organism. The remainder were performed in rats with the exception of two studies which used Guinea pigs (see figure 5). Within these species there were a variety of strains utilized such as BALB/C and Sprague Dawley.

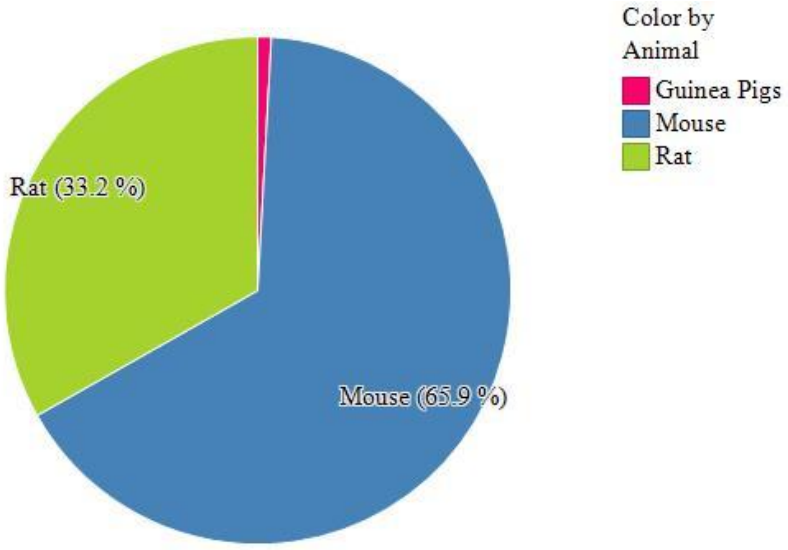

Figure 5. Model Animals Used Across All Three Scaffold Classes. Most experiments were performed in mice.

Collagen cell scaffolds were most often obtained by decellularizing bovine, porcine or rat tissues. Some studies only listed where the collagen was purchased from rather than how it was generated. These studies focused on Collagen I so it is likely that these materials were also derived from decellularized animal tissue. Synthetic and hybrid

scaffolds were most often produced by electrospinning solutions of collagen and polyesters (See figures 6a-c). These studies represent a wide variety of scaffold formulations while adhering to the criteria set forth. The wide range of experimental conditions most likely minimized the chance of sampling bias. The synthetic and hybrid scaffolds were comprised of a variety of polyesters (see figure 7).

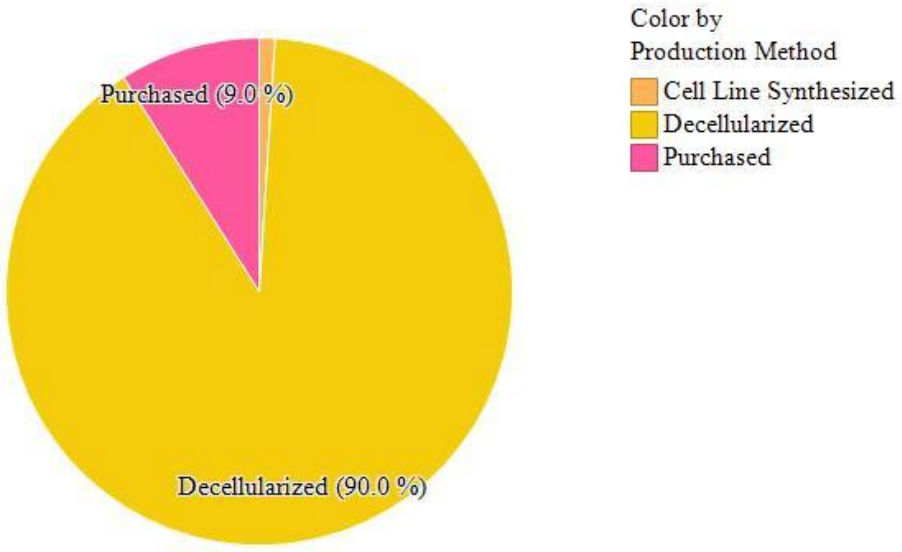

Figure 6a. Fabrication Methods of Collagen Cell Scaffolds. Most collagen cell scaffolds were produced from decellularized animal tissue. The donor tissues varied greatly including bovine skin, rat tail and jellyfish tissue.

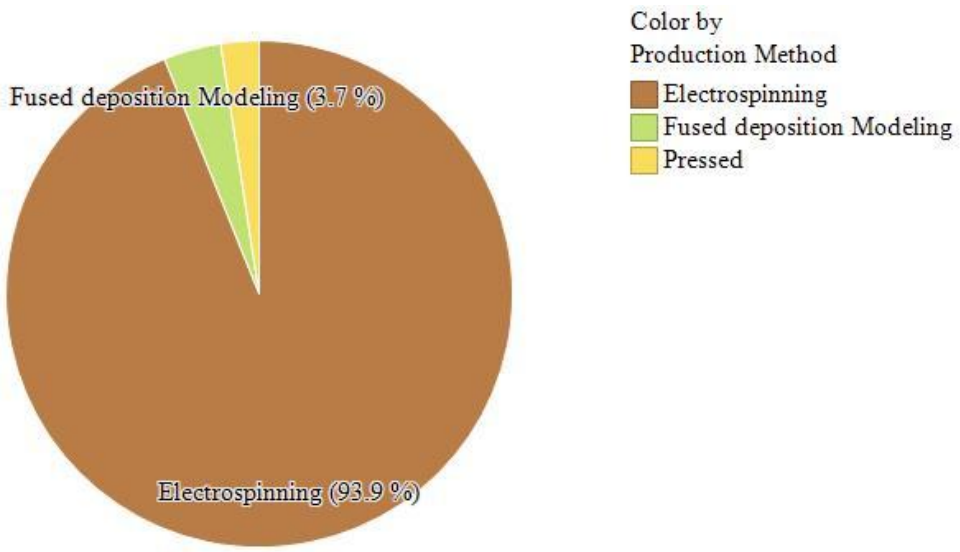

Figure 6b. Fabrication Methods of Polyester Cell Scaffolds. Most polyester scaffolds were produced by electrospinning.

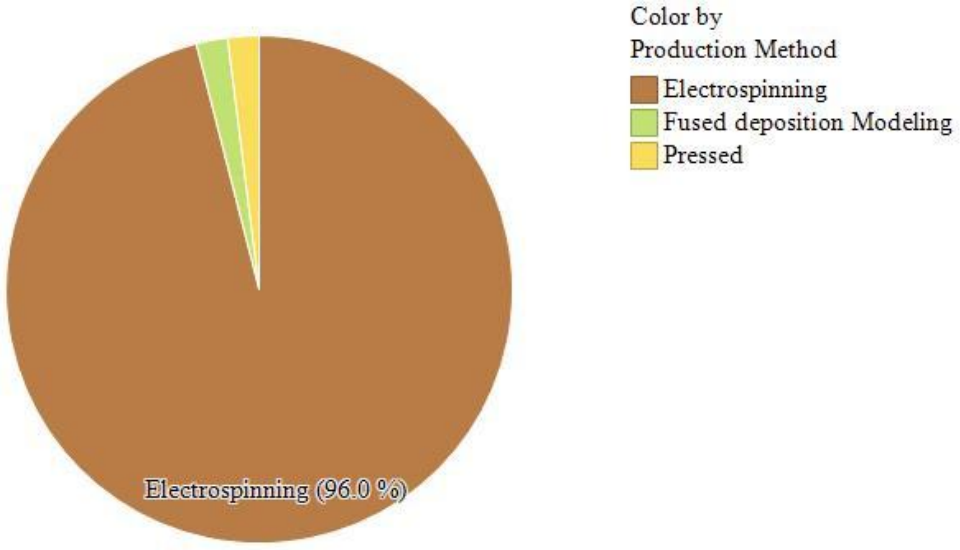

Figure 6c Fabrication Methods of Polyester Collagen Cell Scaffolds. Most polyester collagen scaffolds were produced by electrospinning.

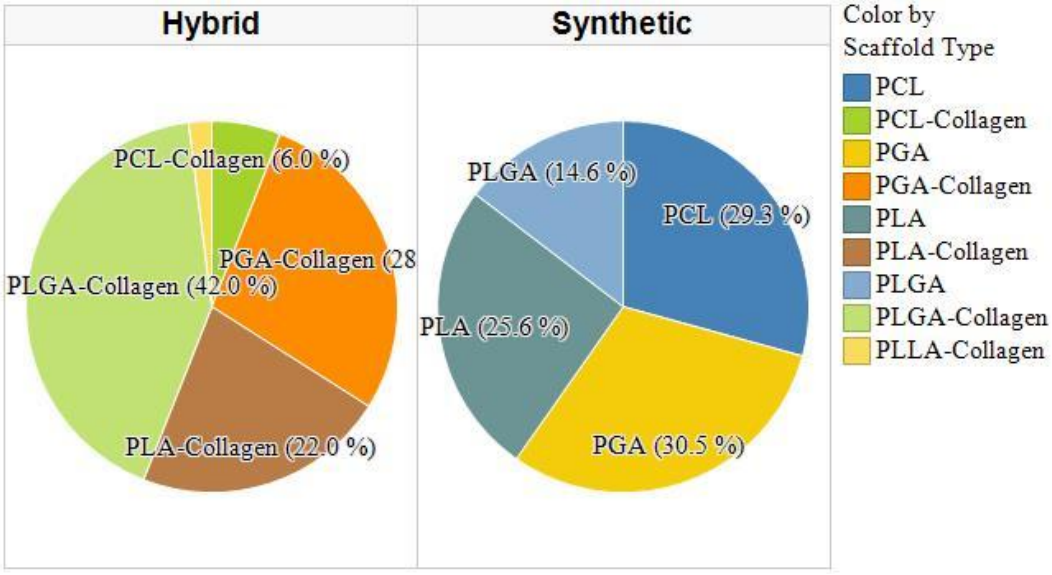

Figure 7 Scaffolds By Polyester Type. A diverse selection of aliphatic homopolymers were utilized for the synthetic scaffolds.

Wound Healing

As discussed above, percent of the wound which had reepithelialized was the most often reported metric in papers reporting epidermal scaffold therapeutics. Though this is not a distinct metric of survival it is indicative of the fact that cells are surviving within the scaffold. As most studies reported the percent of the wound that was repaired over several time points the metric was best visualized as a dot plot over time, (see figure 8).

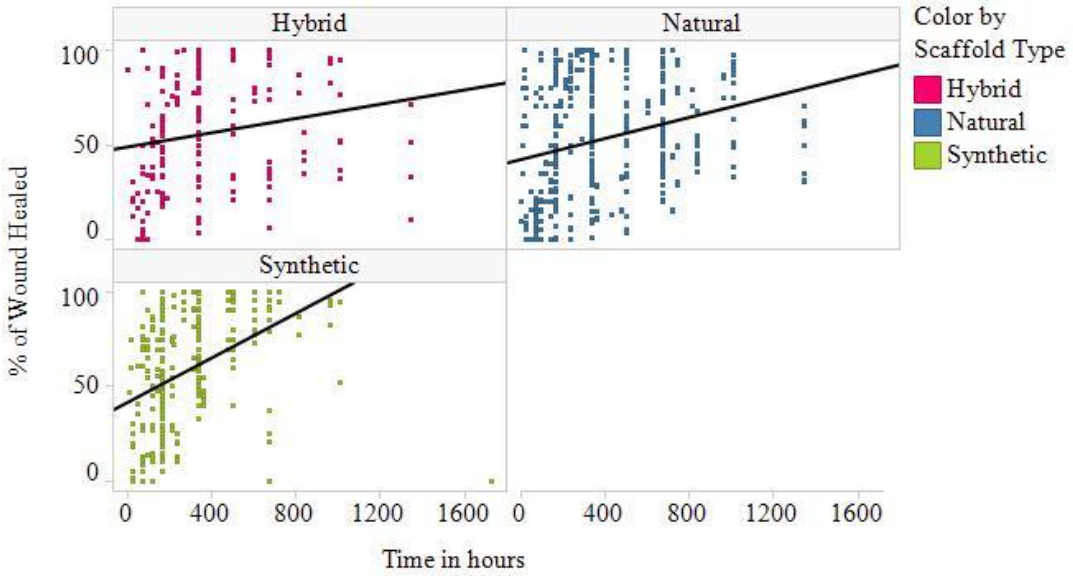

Figure 8. Percent of Wound Repaired vs. Time in Hours by Scaffold Class. The wound repair rate varied greatly both within and across scaffold classes. The best fit lines had the following slopes and goodness of fit, Hybrid (S=0.02, r^2=0.034), Natural (S=0.03, r^2=0.066) Synthetic (S=0.06, r^2=0.171).

The slope of the regression curve here is equal to the percentage healing that occurs per hour. All three scaffold classes display a clear increase in the amount of wound that was repaired over time, though the goodness of fit is always below 0.25 indicating a wide variance in the amount of healing that occurs across studies.

This discrepancy is not completely unexpected, wound dermal repair often accelerates over time and the calculation for percent wound repair is based off of the initial wound size. Reepithelialization will naturally accelerate as the wound's perimeter decreases. But it is still important to understand if these differences are driven my experimental conditions or by the natural processes. The goodness of fit is improved by

switching to a log scale or an exponential regression curve which would better model the acceleration in repair, but the data still indicates that the healing within different studies occurs at different rates (see figure 9). Quadratic and polynomial equations did not fit the data well and were excluded from analysis (data not shown).

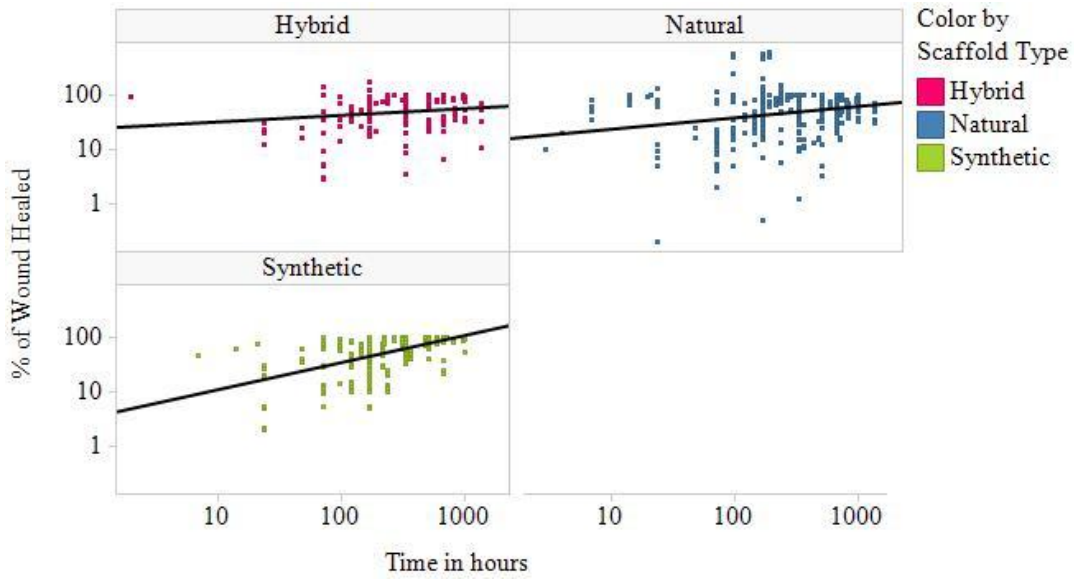

Figure 9. Percent of Wound Repaired vs. Time in Hours by Scaffold Class with Logarithmic Regression. The goodness of fit between time and percent of wound healed improved slightly with a logarithmic curve rather than the linear scale in Figure 8. The best fit lines had the following slopes and goodness of fit, Hybrid (S=0.14, r^2=0.38), Natural (S=0.25, r^2=0.088) Synthetic (S=0.5, r^2=0.299).

To gain a more refined view the healing rate was calculated for individual scaffolds in which the study reported the percent that the wound was repaired over multiple time

periods. The healing rate is still equivalent to percent of skin repaired per hour but limited to a specific scaffold (see figure 10).

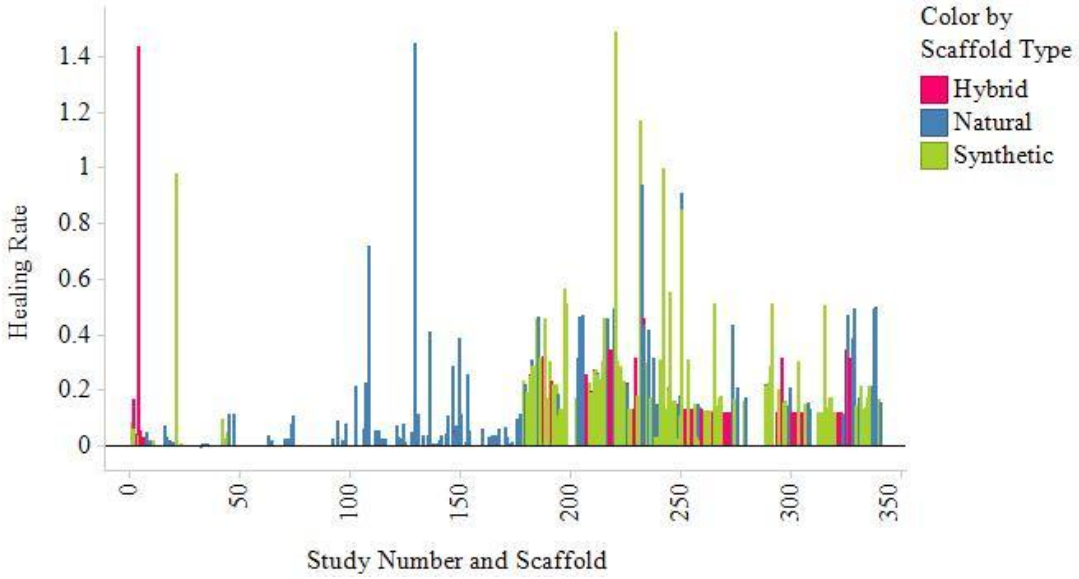

Figure 10. Healing Rate by Study number and scaffold. The healing rates varied even between similar scaffolds applied in the same experiment.

Further subdividing by study numbers shows that the healing rate can vary greatly between scaffolds reported in the same paper which were applied to identical animal cohorts (data not shown). This indicates that the differences in healing are due to experimental conditions rather than biological conditions. It's further worth noting that many of these scaffolds are facilitating healing at a rate greater than the 5%-10%/day, (0.2%-0.4% wound healing/hour), observed in small scale human dermal repair (FRS, 1993).

Lastly the healing rate was compared to the initial surgical area to identify any bias due wound size. One would expect that smaller wounds can be more easily repaired

by the animal's innate regenerative capacity thus representing a smaller contribution from the therapeutic.

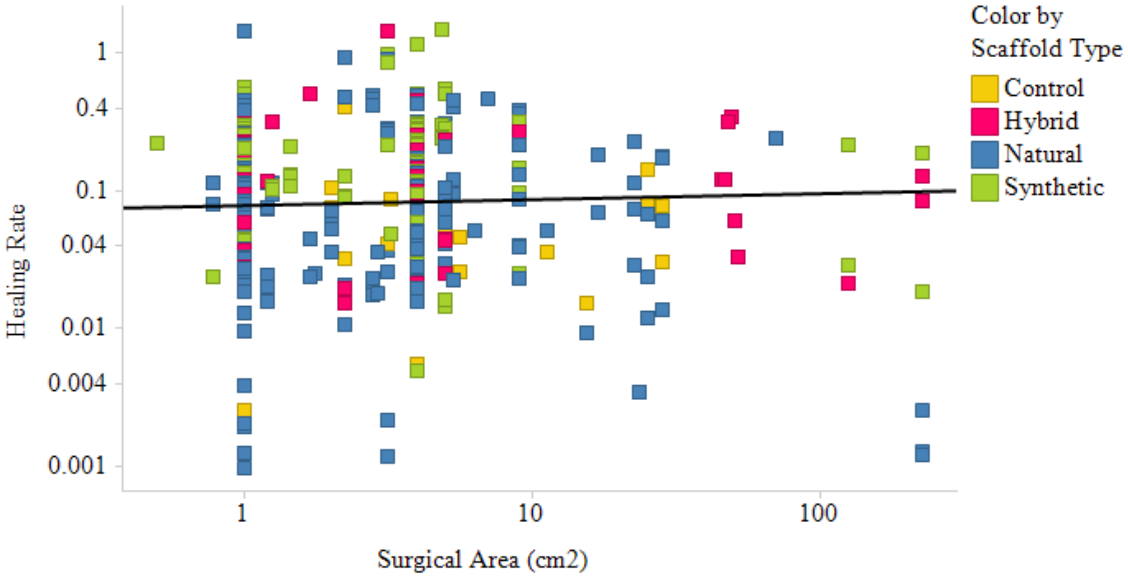

Figure 11. Healing Rate by Surgical Area. Larger surgical area had a weakly positive correlation with healing rate but the relationship is not strong, the goodness of fit was 0.002. The best fit line equation sope is 0.04 and the r^2=0.001 .

Surprisingly, there is little relationship between the area of the wound and the rate of healing (see figure 11). This may indicate that the wounds were so small that the skin would have naturally regenerated without additional aid. Dermal wounds are often broken down into two categories, those that are small enough to reepithelialize into dermis and those that are too large to heal properly, resulting in scars (FRS, 1993). These wound models most closely mimicked smaller wounds which would have healed with

minimal scar tissue. The therapeutics may be more effective at promoting wound repair to avoid infection rather than instigating exceptional regeneration that the body could not have performed itself

Cell scaffold properties which influence wound healing.

The class of cell scaffold was shown to have little impact on the healing rates in natural or hybrid scaffolds but it was shown to be slightly higher in synthetic polyester scaffolds. This difference is statistically significant with a P Value <0.001, indicating that synthetic scaffolds heal faster than hybrid or natural scaffolds (see figure 12 below and Table 11 in the appendix).

Fabrication method also influenced the rate of healing, electrospun scaffolds were shown to heal faster than the natural, decellularized scaffolds. These differences were shown to be significant with P values below 0.0001 (see figure 13 below and Table 12 in the appendix). Some fabrication methods such as collagen obtained by cell culture extrusion were used rarely, N=2. These methods were not considered in further analysis as there is too little data to make

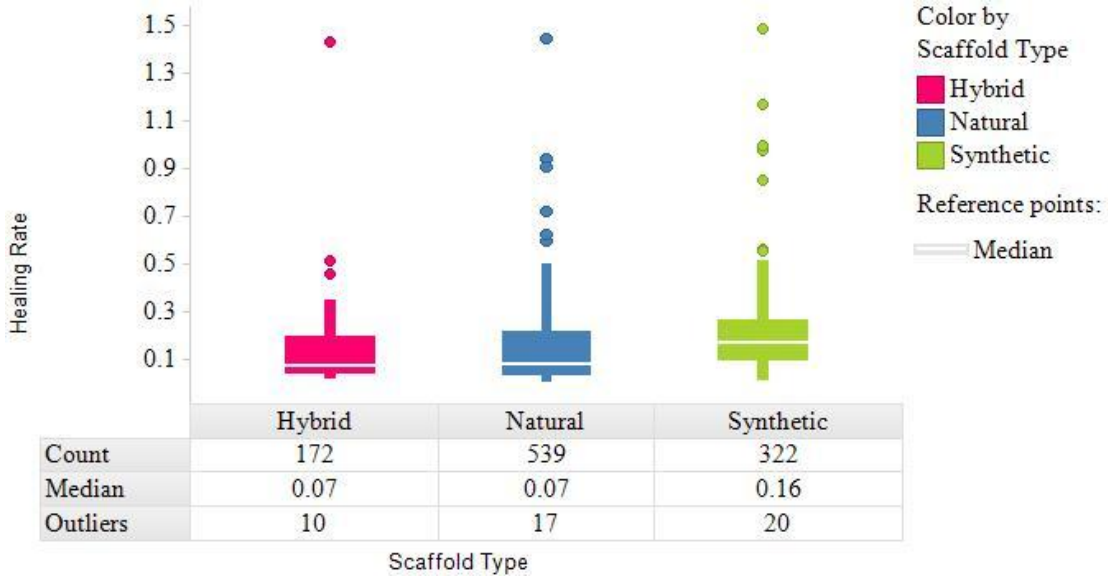

Figure 12. Healing Rate by Scaffold Class. There was no statistical difference in the healing rate of hybrid and natural scaffolds, synthetic scaffolds had higher healing rates than the other two scaffold classes, this difference was found to be statistically significant by ANOVA (see table 11).

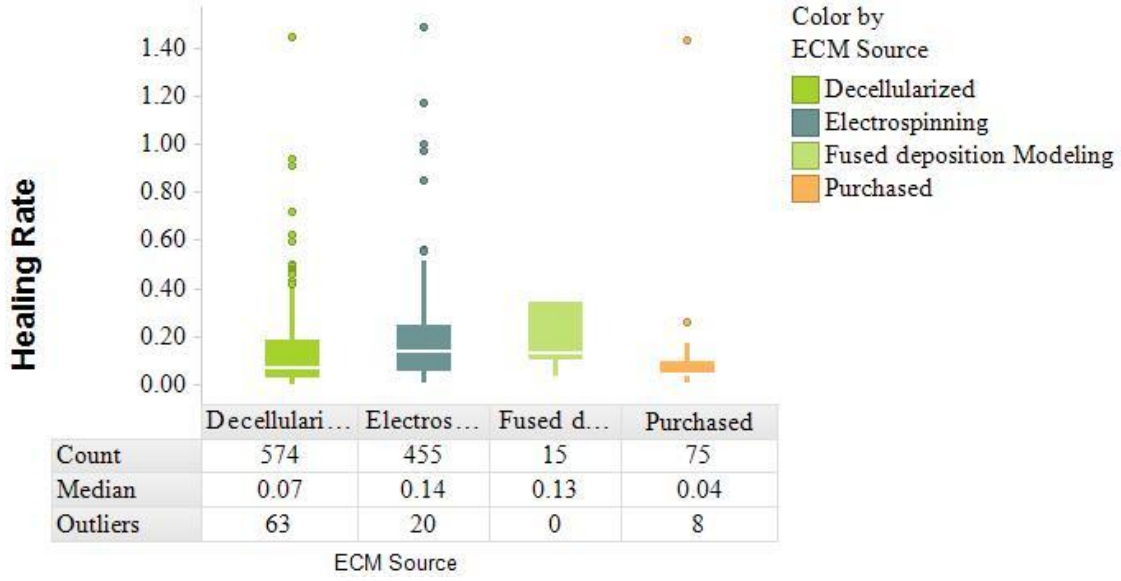

Figure 13. Healing Rate Across Scaffold Fabrication Methods. Only electrospun scaffolds had significantlysignificant higher healing rates than the other fabrication methods when analyzed by ANOVA (see table 12 in the appendix).

The amount of cell seeding was shown to impact the scaffolds healing rate. Direct comparisons between seeded and unseeded scaffolds show that the median healing rate for seeded scaffolds is higher than that of unseeded scaffolds. This difference is statistically significant with a P value <0.0001, (see figure 14).

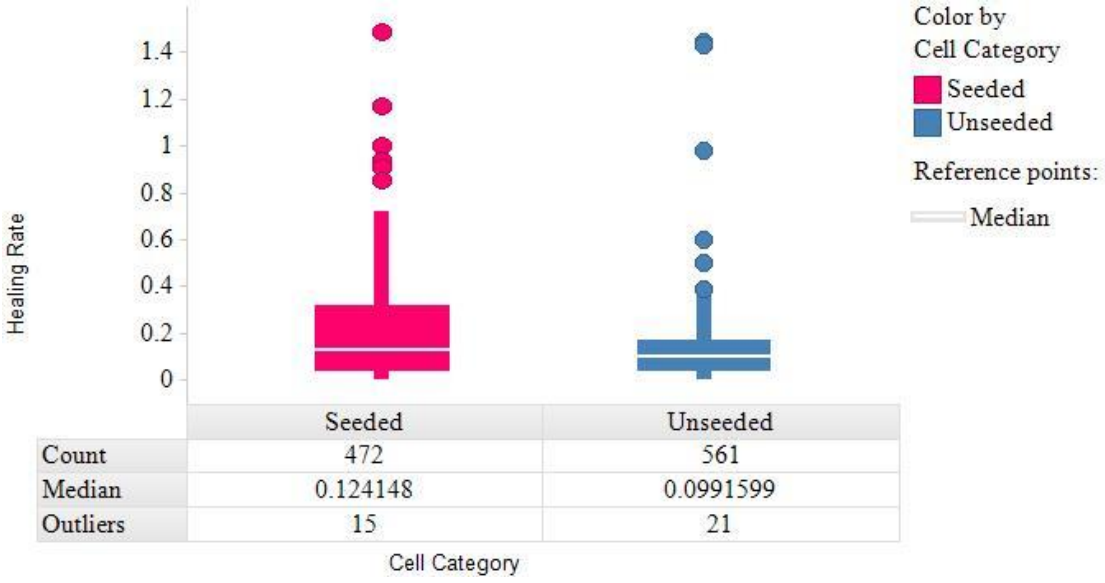

Figure 14. Healing Rates Between Seeded and Unseeded Scaffolds. Seeded scaffolds were had higher healing rates when compared to unseeded scaffolds by a T-test.

Lastly, the rate of wound healing was compared to the amount of cells included with the scaffold. Higher quantities of cells produce a higher healing rate (see figure 15).

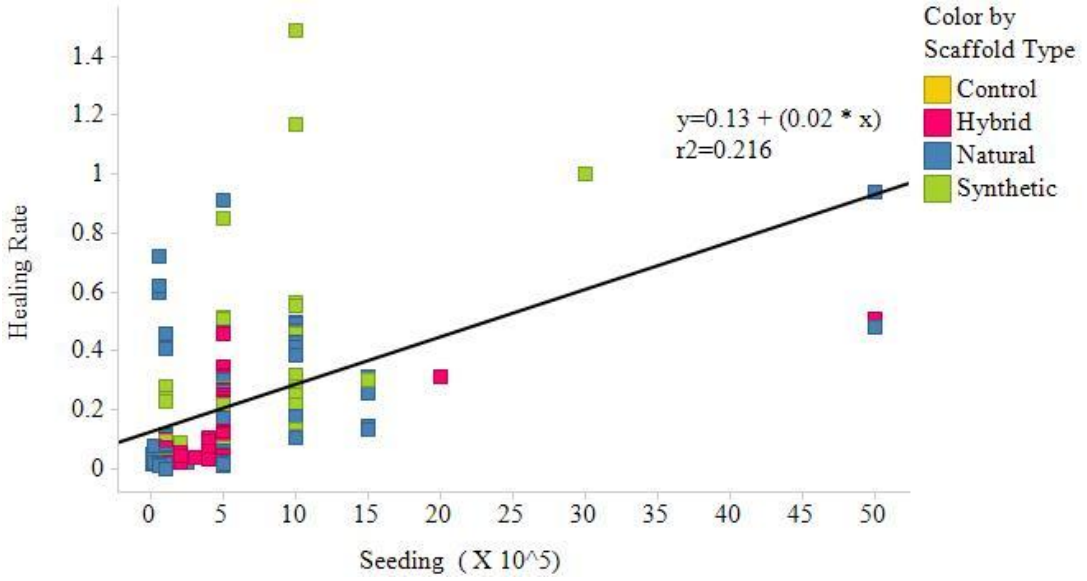

Figure 15. Healing Rate by Number of Seeded Cells. The number of seeded cells was found to positively correlate with the scaffolds healing rate across all three scaffold types but the goodness of fit was relatively small, r^2=0.216.

Together this data indicates that, polyester, electrospun seeded, scaffolds all have higher levels of wound healing and the amount of cells seeded with the scaffold is proportional with the healing rate. However, there is one group of experiments which may skew this data. Some polyester scaffolds have abnormally high healing rates. These studies all contained early measurements, performed within the first 48 hours, during which time there was minimal wound repair, the typical levels of reepithelialization from later time points are exaggerated by comparison. This likely increased the slope of the wound repair resulting in a higher healing rate without an actual increase in

reepithelialization. Because the majority of the cells which are electrospun are polyester this group probably skewed both the comparison across scaffold class and fabrication method. Nonetheless, without a biological reason to exclude these data points they were included in further analysis.

Generation of a Tissue Engineering Reepithelialization Calculator

To operate the reepithelialization calculator users input the type of scaffold in question, the fabrication method, and the amount of cells added, number of days since application and the initial wound size. The calculator is based off of the log based equations relating days of scaffold application and wound size as seen in figure 10. Remaining in log space more accurately predicts the exponential healing rate of natural wound repair, namely a slow rate of repair in the first few days followed by an increasing rapid closure as the wound area decreases. By solving for Y these equations produce the percentage of area which should be reepithelialization by the scaffold class at a given time.

$$\%\ \boldsymbol{of\ WR = Log^{-1}(A + (B\ x\ Log_{10}(X)))}$$

Where WR equals wound repair, A equals the y intercept, B equals the slope of the line (the rate of reepithelialization), and X equals the time since the therapeutic was applied in hours. Having calculated the percent of wound repair the effect of the fabrication is then calculated. The effect of fabrication was calculated using the healing

rate, or percent of the wound healed per hour, (see Table 12 in the appendix, page 98). The difference between the average amount of wound repair and the affect of the fabrication can be added to the % wound repair calculated above. The values for each scaffold class are displayed in Table 4 below.

Table 4		
Rate of reepithelialization by scaffold type		
A is the Y intercept and B is the percentage of wound healed per hour, both by scaffold type.		
	A	B
Natural Scaffolds (Collagen)	0.94	0.24
Synthetic Scaffolds (Polyesters)	0.52	0.51
Hybrid Scaffolds (Collagen-polyester hybrids)	0.98	0.28

Affect of fabrication $= \%\, \boldsymbol{WR}\ \frac{\boldsymbol{AH}}{\boldsymbol{AI}} + \Delta\, \boldsymbol{in\ healing\ rate}\ \frac{\frac{\boldsymbol{AH}}{\boldsymbol{AI}}}{\boldsymbol{TH}}\ \boldsymbol{x\ TH}$ d

Where WR equals wound repair, AH equals the area healed, AI equals the area injured and TH equals the time healed. As only electrospun scaffolds have been found to deviate from the average wound repair rate natural scaffolds do not need to be taken into account.

Δ ***in healing rate*** $=$ Wound rate of electrospun scaffolds- Average Wound rate

The effect of cell seeding was already calculated as a continuous variable using linear regression comparing the time since therapeutic application in hours and the healing rate, see figure 15. As with the effect of fabrication this number can be multiplied by the time since application in hours and added to the previous measurements. Taking the sum of all three metrics produces the final % of wound which has been healed.

$$\% \, \boldsymbol{of \; wound \; healed} = \boldsymbol{SSWR} + \Delta \boldsymbol{WRF} + \Delta \boldsymbol{WRCS}$$

Where SSWR equals the scaffold specific wound repair, WRF equals the wound repair from fabrication and WRCS equals the wound repair from cell seeding.

Wound Reepithelialization Calculator Precision

Ten papers for each of the scaffold classes were randomly selected to serve as a test data set so that the precision of the wound reepithelialization calculator could be assessed. The calculator was a poor predictor of actually reepithelialization, the difference between expected and observed results could vary as much as 40%. The goodness of fit across the different scaffold classes was ordered identically to goodness

of fit calculated for the rate of wound healing computed in the initial data set. Polyester scaffolds were most closely related to their expected reepithelialization rates (R^2=0.151) than collagen polyester (R^2= 0.096) or collagen (R^2= 0.003) scaffolds. It is likely that the variance seen in both test sets is indicative of inherent variance in the scaffold class.

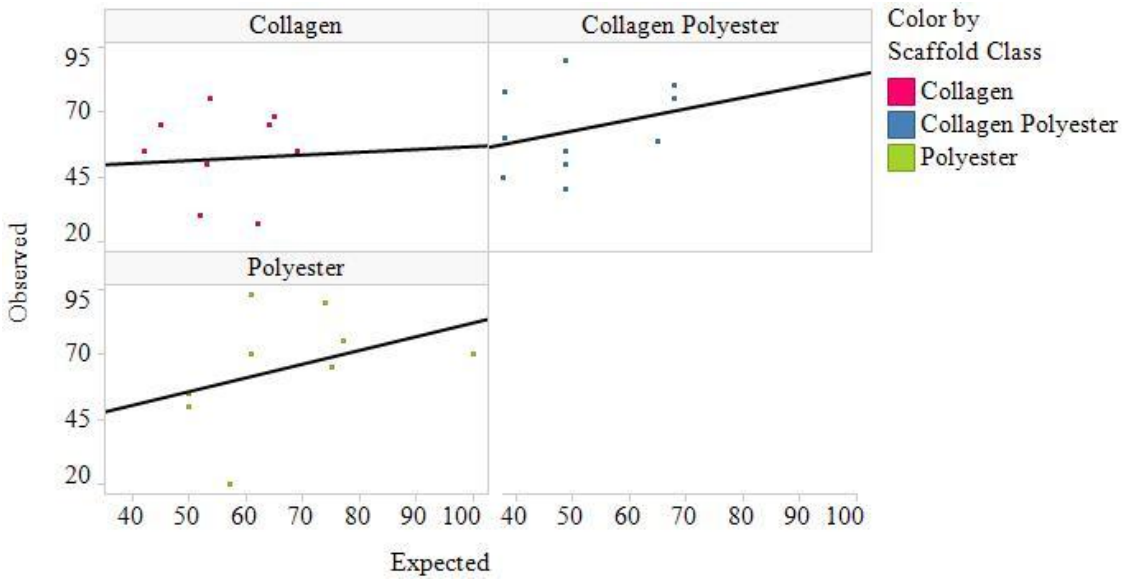

Figure 16. Cell Scaffold Reepithelialization Precision Comparison. The reepithelialization had little concordance between expected and observed wound repair. The best fit lines had the following slopes and goodness of fit, Collagen (S=0.11, r^2=0.003), Collagen Polyester (S=0.43, r^2=0.096) Polyester (S=0.53, r^2=0.151).

The cell scaffold reepithelialization calculator is available for public use at https://drive.google.com/open?id=0B2qtpQ_lfNl8SHBFbjZwaTBSSUE.

Chapter IV

Discussion

After comparing data from over 400 scaffolds the dermal reepithelialization calculator was unable to accurately predict the reepithelialization rates for the 30 test studies. There is little comparison to be made across these different therapeutics other than that the amount of healing increased over time. Healing rates and levels seem to be vastly different across scaffolds and studies. This discrepancy could come from several sources. First the scaffold metrics examined in this study may not have been the most important factors in wound repair. There are many factors which are known to influence cell-surface interactions which were not reported here, tensile strength, contact angle, stiffness, and purity to name a few. More broadly, many of the studies did not report basic information which would be helpful in understanding the scaffolds that were utilized. For example many failed to discuss the size of the wound produced in the animals. Comparing across one of these unaccounted metrics may produce an unobserved symmetry.

The second option is that there is some additional factor producing discrepant results that is only apparent when compared across scaffolds. Most studies only compare the scaffold of interest to a negative control animal model without any additional standard. Perhaps there is some condition which is cofounding wound repair but is not a

part of normal reporting and QC. One such factor could be the scaffold source. Collagen scaffolds in particular are taken from different tissues (rat tail, intestine, cadaverous skin), from different subjects and ages, yet they are treated as identically. We know that cells respond differently in such a wide range of tissues so it follows that wound repair would operate differently in such different scaffolds. This could be one reason why the collagen scaffolds were found to be the most discrepant. It could be that the natural scaffolds are inherently more complex and diverse than the artificial polyester scaffolds or the intermediate composite scaffolds.

The third possible explanation is that the wound repair that is being observed is chiefly due to the animal model and the contributions of the scaffold are positive but minimal. This is supported by considering how long a biodegradable scaffold could have an effect. Some studies have indicated that as little as 3% of the collagen scaffold may be intact after 14 days in vivo (Helary et al., 2010). Many of the scaffolds utilized in this paper have less than 50% reepithelialization by this point so if a scaffold has had an effect it would have needed to occur during the initial wound stabilization and inflammation period. ECM remodeling is a key feature in the repair pathways of host tissue, collagen scaffolds would be especially vulnerable to the expressed MMPs and their inhibitors, TIMPs. This line of questioning implicitly challenges the roll of a cell scaffold. The tissue engineering triad has a tacit assumption that a scaffolds role is to provide a foundation for the cells until the host tissue is able to complete repairs. A better perspective may be that a cell scaffold is most efficacious as a homeostatic stabilizer rather than an actual tissue.

The animals and cells used in these papers also varied greatly strain, age, wound location and animal health would all be expected to impact the rate of wound repair. Similarly the cell passage number, culture medium and handling would impact the survival and durability of seeded cells. Large scale studies have already demonstrated that rodent models can produce vastly different results than human studies which may speak to why many of these therapeutics did not progress to clinical use (Perel et al., 2007; Seok et al., 2013).

While the imprecision of the reepithelialization calculator limit the scope of conclusions to the studies discussed it does emphasize the need for greater standardization. Without common results it is impossible to optimize design or selectively choose the scaffold for the tissue and need. The gap between laboratory tissue engineering therapeutics and clinical tissue engineering therapeutics is often discussed. This work outlines one contributing factor. The lack of relationships between similar therapeutics limits the utility of any given study, effectively making each scaffold an isolated N of one. Future work in tissue engineering would be greatly enhanced by shifting the paradigm away from one of experiments toward a unified system of evaluation.

Appendix

Additional Tables

Table 2

The ECM vs. Cell Scaffolds During the Repair Process.

Stages of wound repair by cellular matrix type.

	1. Homeostatic Disruption	2. Inflammatory Response	3. Repair	4. Remodeling
ECM contribution	The ECM binds platelets blocking pathogens and preventing blood loss.	ECM disruption amplifies the inflammatory response	Extruded ECM stabilizes the wound allowing for cells such as keratinocytes in the skin to move in and fill the abscess.	The ECM is realigned after the less regulated extrusion which occurred during the repair process.
Cell Scaffold Contribution	The body's initial response to damage, cell scaffolds are rarely applied at this stage of wound repair.	Cell Scaffolds can stabilize the wound limiting excessive inflammation and fibrosis.	The ability of the cell scaffold to recruit cells for repair is crucial for enhanced wound repair. (N. -T. Dai, Williamson, Khammo, Adams, & Coombes, 2004)	A scaffolds ability to remodel influences the amount of fibrosis and scaring. (Rosso et al., 2005)

Note: Information compiled from Stroncek et al. unless otherwise noted (Stroncek & Reichert, 2008).

Published data used for analysis

Table 5

Papers with Collagen Scaffolds Used for Analysis.

	Paper Name	First Author	Journal	Year	Reference
1	Evaluation of a multi-layer adipose-derived stem cell sheet in a full-thickness wound healing model	Yen-Chih Lin	Acta Biomateriala	2013	(Lin et al., 2013)
2	Improved cellularization and angiogenesis using collagen scaffolds chemically conjugated with vascular endothelial growth factor	Qifen He	Acta Biomaterialia	2011	(He et al., 2011)
3	The roles of knitted mesh-reinforced collagen-chitosan hybrid scaffold in the one-step	Wang Xingang	Acta Biomaterialia	2013	(Xingang Wang et al., 2013)

	repair of full thickness skin defects in rats				
4	Thiol-ene Michael-type formation of gelatin/poly(ethylene glycol) giomatrices for three-dimensional mesenchymal stromal/stem cell administration to cutaneous wounds	Kedi Xu	Acta Biomaterialia	2013	(Xu et al., 2013)
5	Enhancing repair of full-thickness excisional wounds in a murine model: Impact of tissue-engineered biological dressings featuring human differentiated adipocytes.	Pascal Morissette Martin	Acta Biomaterialia	2015	(Morissette Martin et al., 2015)

6	Engineering a vascularized collagen-β-tricalcium phosphate graft using an electrochemical approach	Yunqing Kang	Acta Biomaterialia	2015	(Y, N, A, J, & Y, 2015)
7	A novel hydrogel-collagen composite improves functionality of an injectable extracellular matrix	R. hartwell	Acta Biomaterialia	2011	(Hartwell et al., 2011)
8	Evaluation of Commonly Used Temporary Skin Dressings and a Newly Developed Collagen Matrix for Treatment of Superficial Wounds	Manuel Held	Advances in Skin & Wound Care	2015	(Held et al., 2015)
9	Effects of Human Adipose-derived Stem cells on Cutaneous Wound Healing in	Seung Ho Lee	Annals of Dermatology	2011	(S. H. Lee, Lee, & Cho, 2011)

	Nude Mice				
10	Efficacy of Novel Collagen/Gelatin Scaffold with Sustained Release of Basic Fibroblast Growth Factor for Dermis-like Tissue Regeneration	Norikazu Kanda	Annals of Plastic Surgery	2012	(Kanda et al., 2012)
11	Accelerated Wound Healing in Healing-Impaired db/db Mice by Autologous Adipose Tissue-Derived Stromal Cells Combined With Atelocollagen Matrix	Masaki Nambu	Annals of Plastic Surgery	2009	(Nambu et al., 2009)
12	Use of Porcine Acellular Dermal Matrix as a	Anil Srivastava	Annals of Surgery	2001	(Anil Srivastava et al., 2001)

	Dermal Substitute in Rats				
13	Cultured Skin Substitutes Reduce Donor Skin Harvesting for Closure of Excised, Full-Thickness Burns	Steven T Boyce	Annals of Surgery	2011	(Desimone et al., 2011)
14	In vitro Studies and Preliminary In vivo Evaluation of Silicified Concentrated Collagen Hydrogels	Martin F. Desimone	Applied Materials & Interfaces	2011	(Desimone et al., 2011)
15	Improvement in wound healing by a novel synthetic collagen-gel dressing in genetically diabetic mice	Dichi Chikazu	Asian Journal of Oral and Maxillofacial Surgery	2010	(Chikazu et al., 2010)
16	Fabrication, quality assurance, and assessment of cultured skin substitutes for treatment of skin wounds	Steven T Boyce	Biochemical Engineering Journal	2004	(Steven T Boyce, 2004)

17	Injectable and Thermosensitive Soluble Extracellular Matrix and Methylcellulose Hydrogels for Stem Cell Delivery in Skin Wounds	Eun Ji Kim	Biomacromolecules	2016	(E. J. Kim, Choi, Kim, Choi, & Cho, 2016)
18	Development of N,O-(Carboxymethyl)chitosan/Collagen Matrixes as Wound Dressing	Ray-Neng Chen	Biomacromolecules	2006	(R.-N. Chen, Wang, Chen, Ho, & Sheu, 2006)
19	Influence of electrospun collagen on wound contraction of engineered skin substitutes	Heather Powell	Biomaterials	2008	(Powell, Supp, & Boyce, 2008)
20	*In vitro* constitution and *in vivo* implantation of engineered skin constructs with sweat glands	Sha Huang	Biomaterials	2010	(Huang, Xu, Wu, Sha, & Fu, 2010)

21	A denatured collagen microfiber scaffold seeded with human fibroblasts and keratinocytes for skin grafting	Margit Kempf	Biomaterials	2011	(Kempf et al., 2011)
22	Enhancement of mesenchymal stem cell angiogenic capacity and stemness by a biomimetic hydrogel scaffold	Kristine Rustad	Biomaterials	2012	(Rustad et al., 2012)
23	Expansion and delivery of human fibroblasts on micronized acellular dermal matrix for skin regeneration	Xiaojun Zhang	Biomaterials	2009	(X. Zhang et al., 2009)
24	Collagen scaffolds derived from a marine source and their biocompatibility	Eun Song	Biomaterials	2006	(Song, Yeon Kim, Chun, Byun, & Lee, 2006)

25	Tissue-engineered dermo-epidermal skin grafts prevascularized with adipose-derived cells	Agnieszka Klar	Biomaterials	2014	(Klar et al., 2014)
26	The effect of cross-linking of collagen matrices on their angiogenic capability	Chang Yao	Biomaterials	2008	(Yao, Markowicz, Pallua, Noah, & Steffens, 2008)
27	Promoted growth of murine hair follicles through controlled release of vascular endothelial growth factor	Makoto Ozeki	Biomaterials	2002	(Ozeki & Tabata, 2002)
28	In vivo promoted growth of mice hair follicles by the controlled release of growth factors	Makoto Ozeki	Biomaterials	2003	(Ozeki & Tabata, 2003)
29	Mitigation of hypertrophic scar contraction via an elastomeric	Elizabeth R. Lorden	Biomaterials	2015	(Lorden et al., 2015)

	biodegradable scaffold				
30	Towards development of a dermal rudiment for enhanced wound healing response.	Yolanda Garcia	Biomaterials	2008	(Garcia, Wilkins, Collighan, Griffin, & Pandit, 2008)
31	The Influence of Pancreas-derived stem cells on scaffold based skin regeneration	H. Salem	Biomaterials	2009	(Salem et al., 2009)
32	Evaluation of dense collagen matrices as medicated wound dressing for the treatment of cutaneous chronic wounds	Christophe Helary	Biomaterials Science	2015	(Helary et al., 2015)
33	Human mesenchymal stem cells successfully improve skin-substitute wound healing	H. Nakagawa	British Journal of Dermatology	2005	(Nakagawa, Akita, Fukui, Fujii, & Akino, 2005)

34	Evaluation of dermal-epidermal skin equivalents ('composite-skin') of human keratinocytes in a collagen-glycosaminoglycan matrix (integra artificial skin)	Michael Kremer	British Journal of Plastic Surgery	2000	(Kremer, Lang, & Berger, 2000)
35	Fibroblasts improve performance of cultured composite skin substitutes on athymic mice	Gulsun Erdag	Burns	2008	(Erdag & Sheridan, 2004)
36	The diamond CO_2 laser as a method of improving the vascularisation of a permanent collagen implant	T.M. MacLeod	Burns	2004	(Macleod, Sarathchandra, Williams, Sanders, & Green, 2004)

37	A potential skin substitute constructed with hEGF gene modified HaCaT cells for treatment of burn wounds in a rat model	Da-hai Hu	Burns	2012	(D. Hu et al., 2012)
38	Combined use of a collagen-based dermal substitute and a fibrin-based cultured epithelium: a step toward a total skin replacement for acute wounds	Beatrice Mis	Burns	2004	(Mis, Rolland, & Ronfard, 2004)
39	Matriderm® versus Integra®: A comparative experimental study	Joerg Schneider	Burns	2009	(Schneider et al., 2009)

40	Experimental study on repairing of nude mice skin defects with composite skin consisting of xenogeneic dermis and epidermal stem cells and hair follicle dermal papilla cells	Shao-Hai Qi	burns	2008	(Qi et al., 2008)
41	Mesenchymal stem cells combined with an artificial dermal substitute improve repair in full-thickness skin wounds	Dilmar Leonardi	Burns	2005	(Nakagawa et al., 2005)
42	Acceleration of wound healing in acute full-thickness skin wounds using a collagen-binding peptide with an affinity for MSCs	Huili Wang	Burns & Truama	2014	(H. Wang et al., 2014)

43	Mesenchymal Stem cells Improve Wound Healing In Vivo via Early Activation of Matrix metalloproteinase-9 and Vascular Endothelial Growth Factor	Chul Han Kim	Cell Therapy & Organ Transplatation	2005	(Nakagawa et al., 2005)
44	In vivo Evaluation of Gelatin/Hyaluronic Acid Nanofiber as Burn-Wound Healing and Its Comparison with ChitoHeal Gel	Bahman Ebrahimi-Hosseinzadeh	Fibers and Polymers	2016	(Ebrahimi-Hosseinzadeh et al., 2016)
45	Microstructure, heological and wound healing properties of collagen-based gel from cuttlefish skin	Mourad Jridi	International Journal of Biological Macromolecules	2015	(Jridi et al., 2015)

46	Drug loaded composite oxidized pectin and gelatin networks for accelerated wound healing	Mythili Tummalapalli	International Journal of Pharmaceutics	2016	(Tummal apalli et al., 2016)
47	Accelerating full thickness wound healing using collagen sponge of mrigal fish (Cirrhinus cirrhosus) scale origin.	Pallabi Pal	Interrnational Journal of Biological Macromolecules	2016	(Pal et al., 2016)
48	Glucose Oxidase Incorporated Collagen Matrices for Dermal Wound Repair in Diabetic Rat Models: A Biochemical Study	V. Arul	Journal of Biomaterials Applications	2011	(V. Arul et al., 2011)
49	Effects of bFGF incorporated into a gelatin sheet on wound healing	Michiyo Miyoshi	Journal of Biomaterials Science Polymer Edition	2005	(Miyoshi et al., 2005)

50	Overexpression of Vascular Endothelial Growth Factor Accelerates Early Vascularization and Improves Healing of Genetically Modified Cultured Skin Substitutes	Dorothy Supp	Journal of Burn Care & Rehabilitation	2002	(Supp & Boyce, 2002)
51	A Newly Designed Collagen-Based Bilayered Scaffold For Skin Tissue Regeneration	Guhan Uzunalan	Journal of Composites and Biodegradable Polymers	2013	(Yıldız Technical University, Uzunalan, Ozturk, Dincer, & Tuzlakoglu, 2013)
52	Mesenchymal stem cells delivered in a microsphere-based engineering skin contribute to cutaneous wound healing and sweat gland repair	Sha Huang	Journal of Dermatological Science	2012	(Huang et al., 2012)
53	Effects of a collagen matrix containing postaglandin	Ichiro Ono	Journal of Dermatological Science	2001	(Ono, Zhou, & Tateshita, 2001)

	E1 on wound contraction				
54	Topical Nutrients Promote Engraftment and Inhibit Wound Contraction of Cultured Skin Substitutes in Athymic Mice	Steven T. Boyce	Journal of Investigative Dermatology	1995	(Steven T. Boyce, Supp, Harriger, Greenhalgh, & Warden, 1995)
55	Nanofibers prepared by needless electrospinning technology as scaffolds for wound healing	Michal Dubsky	Journal of Material Science: Materials in Medicince	2012	(Dubský et al., 2012)
56	Protease-sensitive atelocollagen hydrogels promote healing in a diabetic wound model	Giuseppe Tronci	Journal of Materials Chemistry B	2016	(Tronci et al., 2016)
57	Nanofibers prepared by needleless electrospinning technology as scaffolds for wound healing	Michal Dubsky	Journal of Materials Science: Materials in Medicine	2012	(Dubský et al., 2012)

58	Higher numbers of autologous fibroblasts in an artificial dermal substitute improve tissue regeneration and modulate scar tissue formation	Evert N. Lamme	Journal of Pathology	2000	(Lamme, Van Leeuwen, Brandsma, Van Marle, & Middelkoop, 2000)
59	Collagen-based wound dressing for doxycycline delivery in-vivo evaluation in an infected excisional wound model in rats	Natarajan Adhirajan	Journal of Pharmacy and Pharmacology	2009	(Adhirajan, Shanmugasundaram, Shanmuganathan, & Babu, 2009)
60	Wound Healing on Athymic Mice With Engineered Skin Substitutes Fabricated with Keratinocytes Harvested from an Automated Bioreactor	Balaji Kalyanaraman	Journal of Surgical Research	2009	(Kalyanaraman & Boyce, 2009)

61	Long-Term Outcome of Xenogenic Dermal Matrix Implantation in Immunocompetent Rats	Evangeline Z. DeSagun	Journal of Surgical Research	2001	(DeSagun, Botts, Srivastava, Hanumadass, & Walter, 2001)
62	Stimulatory Effect of Autologous Adipose Tissue-Derived Stromal cells in an Atelocollagen Matrix on Wound Healing in Diabetic db/db Mice	Masaki Nambu	Journal of Tissue Engineering	2011	(Nambu et al., 2011)
63	Targeted delivery of adipose-derived stem cells via acellular dermal matrix enhances wound repair in diabetic rats	Chunlei Nie	Journal of Tissue Engineering and Regenerative Medicine	2015	(Nie et al., 2015)
64	Comparison of Dermal Substitutes in Wound Healing Utilizing a Nude Mouse Model	Anh-Tuan N. Truong	Journal of Wounds and Burns	2005	(Truong, Kowal-Vern, Latenser, Wiley, & Walter, 2005)

65	Acceleration of diabetic wound healing with chitosan-crosslinked collagen sponge containing recombinant human acidic fibroblasts growth factor in healing-impaired STZ diabetic rats	Wei Wang	Live Sciences	2008	(W. Wang et al., 2008)
66	A therapeutic approach for diabetic wound healing using biotinylated GHK incorporated collagen matrices	Vadivel Arul	Live Sciences	2007	(Vadivel Arul, Kartha, & Jayakumar, 2007)
67	Bacterial Cellulose/Collagen Hydrogel for Wound Healing	Paula Rodrigues	Materials Research	2016	(Moraes et al., 2016)

68	Simultaneous dual syringe electrospinning system using benign solvent to fabricate nanofibrous P(3HB-co-4HB)/collagen peptides construct as potential leave-on wound dressing	S. Vigneswari	Materials Science and Engineering C	2016	(Vigneswari, Murugaiyah, Kaur, Abdul Khalil, & Amirul, 2016)
69	Influence of radiation crosslinked carboxymethyl-chitosan/gelatin hydrogel on cutaneous wound healing	Xin Huang	Materials Science and Engineering C	2013	(X. Huang et al., 2013)
70	Enhancement of skin wound healing with decellularized scaffolds loaded with hyaluronic acid and epidermal growth factor	Zhongchun Su	Materials Science and Engineering: C	2014	(Su et al., 2014)

71	Extracellular matrix-derived products modulate endothelial and progenitor cell migration and proliferation *in vitro* and stimulate regenerative healing *in vivo*	Ekaterina Vorotnikova	Matrix Biology	2010	(Vorotnikova et al., 2010)
72	A Collagen-based Scaffold Delivering Exogenous MicroRNA-29B to Modulate Extracellular Matrix Remodeling	Michael Monaghan	Molecular Therapy	2014	(Monaghan, Browne, Schenke-Layland, & Pandit, 2014)
73	Complete Horizontal Skin Cell Resurfacing and Delayed Vertical Cell Infiltration into Porcine Reconstructive Tissue Matrix Compared to Bovine Collagen Matrix and Human	Ursula Mirastschijski	Plastic and Reconstructive Surgery	2013	(Mirastschijski, Kerzel, Schnabel, Strauss, & Breuing, 2013)

	Dermis				
74	Novel Biodegradable Porous Scaffold Applied to Skin Regeneration	Hui-Min Wang	PLOS One	2013	(D. W.-C. Chen, Liao, Liu, & Chan, 2012)
75	Diabetic wound regeneration using peptide-modified hydrogels to target re-epithelialization	Yun Xiao	PNAS	2016	(Xiao et al., 2016)
76	Design and development of a piscine collagen blended pullulan hydrogel for skin tissue engineering	Iswariya S.	RSC Advances	2016	

77	Clinical Evaluation of an Alleogeneic Cultured Dermal Substitute Composed of Fibroblasts within a spongy Collagen Matrix	Naoto Yamada	Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery	1999	(Yamada, Uchinuma, & Kuroyanagi, 1999)
78	PLLA-collagen and PLLA-Gelatin Hybrid scaffolds with funnel-like porous structure for skin tissue engineering	Hongxu Lu	Science and Technology of Advanced Materials	2012	(Lu, Oh, Kawazoe, Yamagishi, & Chen, 2012)
79	In vivo biocompatibility, vascularization, and incorporation of Integra dermal regenerative template and flowable wound matrix	Thomas Spater	Society For Biomaterials	2016	(Später, Frueh, Metzger, Menger, & Laschke, 2016)

80	Bioprinted Amniotic Fluid-Derived Stem Cells Acclerate Healing of Large Skin Wounds	Skardal Aleksander	Stem Cells Translational Medicince	2012	(Skardal et al., 2012)
81	Capillary Force Seeding of Hydrogels for Adipose-Derived Stem Cell Delivery In Wounds	Garg Ravi K	Stem Cells Translational Medicine	2014	(Garg et al., 2014)
82	The Wound-healing effect of a glycoprotein fraction isolated from aloe vera	S-W. Choi	The British Hournal of Dermatology	2001	(S.-W. Choi et al., 2001)
83	The role of oxidised regenerated cellulose/collagen in wound repair: effects in vitro on fibroblast biology and in vivo in a model of compromised healing	Jeffrey Hart	The International journal of Biochemistry & Cell Biology	2002	(Hart et al., 2002)

84	Spongy matrix of hyaluronic acid and collagen aqs dermal substitute:evlauation in an animal test	Kentaro Kubo	The Japanese Society for artificial orgnas	2003	(Kubo & Kuroyanagi, 2003)
85	In Vivo Model of Wound Healing Based on Transplanted Tissue-Engineered Sin	David Geer	Tissue Engineering	2004	(Geer, Swartz, & Andreadis, 2004)
86	A Rapid Fabricated Living Dermal Equivalent for Skin Tissue Engineering: An In Vivo Evaluation in an Acute Wound Model	Michael Ananta	Tissue Engineering Part A	2011	(Michael Ananta, Brown, & Mudera, 2011)
87	A Rapid Fabricated Living Dermal Equivalent for Skin Tissue Engineering: An *In Vivo* Evaluation in an Acute Wound	Michael Ananta	Tissue Engineering Part A	2016	(Ananta et al., 2011)

	Model				
88	Functional Bilayered Skin Substitute Constructed by Tissue-Engineered Extracellular Matrix and Microsphere-Incorporated Gelatin Hydrogel for Wound Repair	Sha Huang	Tissue Engineering Part A	2009	(Huang et al., 2009)
89	*In Vivo* Assessment of Printed Microvasculature in a Bilayer Skin Graft to Treat Full-Thickness Wounds	Maria Yanez	Tissue Engineering Part A	2014	(Yanez et al., 2014)
90	Preparation of Collagen/Gelatin Sponge Scaffold for Sustained Release of bFGF	Satoru Takemoto	Tissue Engineering Part A	2008	(Takemoto et al., 2008)

91	Engineered Pullulan–Collagen Composite Dermal Hydrogels Improve Early Cutaneous Wound Healing	Victor Wong	Tissue Engineering Part A	2010	(Wong et al., 2010)
92	Full-Thickness Skin Wound Healing using Human Placenta-Derived Extracellular matrix Containing Bioactive Molecules	Ji Suk Choi	Tissue Engineering Part A	2007	(J. S. Choi, Kim, Yoon, & Cho, 2013)
93	Efficacy of the Controlled Release of Concentrated Platelet Lysate from a Collagen/Gelatin Scaffold for Dermis-Like Tissue Regeneration	Ran Ito	Tissue Engineering Part A	2013	(Ito et al., 2013)

94	Adipose-Derived Stem Cell-Seeded Hydrogel Increase Endogenous Progenitor Cell Recruitment and Neovascularization in Wounds	Revanth Kosaraju	Tissue Engineering Part A	2016	(Kosaraju et al., 2016)
95	Enhanced Angiogenesis in Porous Collagen-Chitosan Scaffolds Loaded with Angiogenin	Haifei shi	Tissue Engineering Part A	2008	(Shi, Han, Mao, Ma, & Gao, 2008)
96	Evaluation of a porcine origin acellular dermal matrix and small intestinal submucosa as dermal replacements in preventing secondary skin graft contraction	Mai Tam	Tissue Engineering: Part A	2015	(MacLeod, Sarathchandra, Williams, Sanders, & Green, 2004)

97	Effective Delivery of stem Cells using an extracellular Matrix Patch Results in Increased Cell Survival and Proliferation and Reduced Scarring in Skin Wound Healing	Mai T. Lam	Tissue Engineering: Part A	2012	(Lam, Nauta, Meyer, Wu, & Longaker, 2012)
98	Cutaneous Wound Healing After Treatment with Plant-Derived Human Recombinant Collagen Flowable Gel	Shani Shilo	Tissue Engineering: Part A	2012	(Shilo et al., 2012)
99	Regulation of cutaneous pigmentation by titration of human melanocytes in cultured skin substitutes grafted to athymic mice	Viki B. Swope	Wound Repair and Regeneration	2013	(Swope, Supp, & Boyce, 2002)

100	Moduclation of scar tissue formation using different dermal regeneration templates in the treatment of experimental full-thickness wounds.	Daniel Drueck	Wound repair and regeneration. The international journal of tissue repair and regeneration	2011	(Druecke et al., 2004)

Table 6					
Papers With Polyester Collagen Scaffolds Used in This Analysis					
1	Gentamicin-Loaded Wound Dressing With Polyvinyl Alcohol/Dextran Hydrogel: Gel Characterization and In Vivo Healing Evaluation	Ma-Ro Hwang	AAPS PharmSciTech	2010	(M.-R. Hwang et al., 2010)
2	In-Situ-Generated Vasoactive Intestinal Peptide Loaded Microspheres in Mussel-Inspired Polycaprolactone Nanosheets Creating Spatiotemporal Releasing Microenvironment to Promote Wound Healing and Angiogenesis	Yuzhen Wang	ACS Applied Materials & Interfaces	2016	(Yuzhen Wang et al., 2016)

3	Polyhydroxybutyrate-co-hydroxyvalerate structures loaded with adipose stem cells promote skin healing with reduced scarring	Alessandra Zonari	Acta Biomaterialia	2015	(Zonari et al., 2015)
4	Three-dimensional hydrogel scaffolds facilitate in vitro self-renewal of human skin-derived precursors	Xinyu Wang	Acta Biomaterialia	2014	(Cai et al., 2014; Xinyue Wang et al., 2014)
5	Application of Poly-L-Lactic Acid Nanosheet as a Material for Wound Dressing	Shimpo Aoki	American Society of Plastic Surgeons	2013	(Aoki et al., 2013)
6	Use of Porcine Acellular Dermal Matrix as a Dermal Substitute in Rats	Anil Srivastava	Annals of Surgery	2001	(Anil Srivastava et al., 2001)
7	Bio-Conjugated Polycaprolactone Membranes: A Novel Wound Dressing	Elijah Zhengyang Cai	Archives of Plastic Surgery	2014	(Cai et al., 2014)
8	The healing eff ect of unrestricted somatic stem cells loaded in collagen-modified nanofibrous PHBV scaffold on full-thickness skin defects	Saeed Heidari Keshel	Artificial Cells, Nanomedicine and Biotechnology	2014	(Keshel et al., 2014)
9	Tissue Engineered Artificial Skin Composed of Dermis and Epidermis	Eun Kyung Yang	Artificial Organs	2000	(E. K. Yang et al., 2000)

10	Effect of Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) Nanofiber Matrices Cocultured With Hair Follicular Epithelial and Dermal cells for Biological Wound Dressing	Insook Han	Artificial Organs	2007	(Han et al., 2007)
11	Promotion of Full-Thickness Wound healing using Epigallocatechin-3-0-Fallate/Poly (Lactic-Co-Glycolic Acid) Membrane as Temporary Wound Dressing	HL Kim	Artificial Organs	2013	(Ruvinov, Leor, & Cohen, 2011)
12	In Vivo Degradation behavior of Photo-Cross-Linked star-Poly(E-caprolactone-co-D,L-Lactide) Elastomers	Brian G. Amsden	BioMacromolecules	2014	(Amsden, Tse, Turner, Knight, & Pang, 2006)
13	Development of Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) Fibers for Skin Tissue Engineering: Effects of Topography, Mechanical, and Chemical Stimuli	Purushothaman Kuppan	BioMacromolecules	2011	(Kuppan, Vasanthan, Sundaramurthi, Krishnan, & Sethuraman, 2011)
14	Polycaprolactone-based fused deposition modeled mesh for delivery of antibacterial agents to infected wounds	Erin Yiling Teo	Biomaterials	2011	(Teo et al., 2011)

15	In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF)	Ji Suk Choi	Biomaterials	2008	(J. S. Choi, Leong, & Yoo, 2008)
16	Biomimetic LBL structured nanofibrous matrices assembled by chitosan/collagen for promoting wound healing	Rong Huang	Biomaterials	2015	(R. Huang et al., 2015)
17	Mitigation of hypertrophic scar contraction via an elastomeric biodegradable scaffold	Elizabeth R. Lorden	Biomaterials	2015	(Lorden et al., 2015)
18	Reduced contraction of skin equivalent engineered using cell sheets cultured in 3D matrices	Kee Woei Ng	Biomaterials	2006	(Ng & Hutmacher, 2006)
19	Tissue-engineered dermo-epidermal skin grafts prevascularized with adipose-derived cells	Agnieszka S. Klar	Biomaterials	2014	(Klar et al., 2014)
20	Biodegradable lysine diisocyanate-based poly(glycolide-co-E-Caprolactone)-urethane network in artificial skin	P. Bruin	Biomaterials	1998	(Bruin, Smedinga, Pennings, & Jonkman, 1990)
21	The enhancement of VEGF-mediated angiogenesis by polycaprolactone scaffolds with surface cross-linked heparin	Shivani Singh	Biomaterials	2011	(Singh, Wu, & Dunn, 2011)

22	Development of biodegradable electrospun scaffolds for dermal replacement	Keith Blackwood	Biomaterials	2008	(Blackwood et al., 2008)
23	Dual delivery of growth factors with coacervate-coated poly(lactic-co-glycolic acid) nanofiber improves neovascularization in a mouse skin flap model	Min Suk Lee	Biomaterials	2017	(M. S. Lee et al., 2017)
24	Surface biofunctional drug-loaded electrospun fibrous scaffolds for comprehensive reparing hypertrophic scars	Liying Chen	Biomaterials	2016	(Cheng et al., 2016)
25	Three types of dermal grafts in rats: the importance of mechanical property and structural design	Chuangang You	BioMedical Engineering Online	2013	(You, Wang, Zheng, & Han, 2013)
26	Fabrication of chitosan-polycaprolactone composit nanofibrous scaffold for simultaneous delivery of ferulic acid and revratol	Balan Poornima	Carbohydrate Polymers	2017	(Poornima & Korrapati, 2017)
27	Neurotrophin-3 Accelerates Wound Healing in Diabetic Mice by Promoting a Paracrin Response in Mesenchymal Stem Cells	Lei Shen	Cell Transplantation	2013	(Shen et al., 2013)

28	Tissue-engineered human skin substitutes developed from collagen-populated hydrated gels: clinical and fundamental applications	F. A. Auger	Cellular Engineering: Bioengineering Of The Skin	1998	(Auger et al., 1998)
29	Characterization of pigmented dermo-epidermal skin substitutes in a long-term in vivo assay	Sophie Bottcher-Haberzeth	Experimental Dermatology	2015	(Böttcher-Haberzeth et al., 2015)
30	Electrospinning of curcumin loaded chitosan/poly (lactic acid) nanofilm and evaluation of its medicinal characteristics	Bhaarathi Dhurai	Frontiers in Material Science	2013	(Dhurai et al., 2013)
31	Platelet-Rich Plamsa-Loaded Poly(D,L-Lactide)-Poly(ethyleneglycol)-Poly(D,L-lactide) Hydrogel Dressing Promotes Full-Thickness Skin Wound Healing in a Roden Model	Manle Qiu	International Journal of Molecular Sciences	2016	(Qiu et al., 2016)
32	Engineering of epidermis skin grafts using electrospun nanofibrous gelatin/ polycaprolactone membranes	Huichuan Duan	International Journal of Nanomedicine	2013	(Duan et al., 2013)
33	EGF and curcumin co-encapsulated nanoparticle/hydrogel system as potent skin regeneration agent	Xiaoling Li	International Journal of Nanomedicine	2016	(X. Li et al., 2016)

34	In situ gel-forming AP-57 peptide delivery system for cutaneous wound healing	Xiaoling Li	International Journal of Pharmaceutics	2015	(X. Li et al., 2015)
35	In vivo Behavior of Poly(E-Caprolactone-co-DL-Lactide/Bioactive Glass Composites in Rat Subcutaneous Tissue	T. Ranne	Journal of Bioactive and compatible Polymers: Biomedical Applications	2007	(Ranne et al., 2007)
36	Thermal dehydration treatment and glutaraldehyde cross-linking to increase the biostability of collagen-chitosan porous scaffolds used as dermal equivalent	Changyou Lie	Journal of Biomaterials Science, Polymer Edition	2003	(Lie et al., 2003)
37	The effect of a Self-Assembling Peptide Nanofiber Scaffold (Peptide) When Used as a Wound Dressing for the Treatment of Deep Second Degree Burns in Rats	Hui Meng	Journal of Biomedical Material Research B: Applied Biomaterials	2008	(Meng, Chen, Ye, Wang, & Zhao, 2009)

38	Long term in vivo degradation and tissue response to photo-cross-linked elastomers prepared from star-shaped prepolymers of poly(E-caprolactone-co-D,L-lactide)	Rafi Chapanian	Journal of Biomedical Materials Research	1999	(Chapanian, Tse, Pang, & Amsden, 2010)
39	In vitro and in vivo degradation behavior of n-HA/PCL-Pluronic-PCL polyurethane composite	Shao-Zhi Fu	Journal of Biomedical Materials Research	2000	(S.-Z. Fu, Meng, Fan, Yang, Lin, et al., 2014)
40	In vivo wound healing and antibacterial performances of electrospun nanofibre membranes	Xin Liu	Journal of Biomedical Materials Research Part A	2010	(X. Liu et al., 2010)
41	The effect of active ingredient-containing chitosan/polycaprolactone nonwoven mat on wound healing: In vitro and in vivo studies	Meng-Yi Bai	Journal of Biomedical Materials Research Part A	2013	(Bai, Chou, Tsai, & Yu, 2014)
42	Accelerated skin wound healing using electrospun nanofibrous mats blended with mussel adhesive protein and polycaprolactone	Bum Jin Kim	Journal of Biomedical Materials Research Part A	2016	(B. J. Kim et al., 2017)

43	Acceleration of dermal wound healing by using electrospun curcumin loaded poly(E-caprolactone)-poly(ethylene glycol)-poly(E-caprolactone) fibrous mats	Shao-Zhi Fu	Journal of Biomedical Materials Research Part B Applied Biomaterials	2014	(S.-Z. Fu, Meng, Fan, Yang, Wen, et al., 2014)
44	Synthesis and wound healing of alternating block polyurethanes based on poly(lactic acid) (PLA) and poly (ethylene glycol) (PEG)	Linjing Li	Journal of Biomedical Materials Research Part B Applied Biomaterials	2016	(L. Li et al., 2016)
45	Morphology, drug release, antibacterial, cell proliferation, and histology studies of chamomile-loaded wound dressing mats based on electrospun nanofibrous poly(ε-caprolactone)/polystyrene blends	Behrooz Motealleh	Journal of Biomedical Materials Research Part B Applied Biomaterials	2014	(Motealleh et al., 2014)
46	Synergetic effect of 3,4-dihydroxy-l-phenylalanine-modified poly(lactic-co-glycolic acid) nanopatterned patch with fibroblast growth factor-2 for skin wound regeneration	Min Suk Lee	Journal of Biomedical Materials Research Part B Applied Biomaterials	2015	(M. S. Lee, La, Park, & Yang, 2015)

47	Xenogeneic Acellular Dermal Matrix as a Dermal Substitute in Rats	Anil Srivastava	Journal of Burn Care & rehabilitation	1999	(A. Srivastava et al., 1999)
48	Comparison of Dermal Substitutes in Wound Healing Utilizing a Nude Mouse Model	Anh-Tuan N. Truong	Journal of Burns and Wounds	2005	(Truong et al., 2005)
49	A Nanoscaffold impregnanted with human wharton's jelly stem cells or It's Secretions Improves Healing of Wounds	Kimberly Tam	Journal of Cellular Biochemistry	2014	(Tam et al., 2014)
50	Characteristics of curcumin-loaded poly (lactic acid) nanofibers for wound healing	Thuy Thi Thu Nguyen	Journal of Material Science	2013	(T. T. T. Nguyen, Ghosh, Hwang, Tran, & Park, 2013)
51	Nanofibers prepared by needless electrospinning technology as scaffolds for wound healing	Michal Dubsky	Journal of Material Science: Materials in Medicince	2012	(Dubský et al., 2012)
52	Polyvinyl alcohol-based hydrogel dressing gellable on-wound via a co-enzymatic reaction triggered by glucose in the wound exudate	Shinji Sakai	Journal of Materials Chemistry B	2013	(Sakai et al., 2013)

53	Chitosan-Poly(caprolactone) Nanofibers for Skin Repair	Sheeny Lan Levengood	Journal of Materials Chemistry B	2017	(Levengood, Erickson, Chang, & Zhang, 2017)
54	Experimental wound dressings of degradable PHA for skin defect repair	Ekaterina Shishatskaya	Journal of Materials Science: Materials in Medicine	2016	(Shishatskaya, Nikolaeva, Vinogradova, & Volova, 2016)
55	Nanofibers prepared by needleless electrospinning technology as scaffolds for wound healing	Michal Dubsky	Journal of Materials Science: Materials in Medicine	2012	(Dubský et al., 2012)
56	Electrospun Membranes of Poly(Lactic Acid) (PLA) Used as Scaffold in Drug Delivery of Extract of Sedum Dendroideum	Larissa Santos	Journal of Nanoscience and Nanotechnology	2013	(Santos et al., 2013)
57	Wound Healing on Athymic Mice With Engineered Skin Substitutes Fabricated with Keratinocytes Harvested from an Automated Bioreactor	Balaji Kalyanaraman	Journal of surgical Research	2009	(Kalyanaraman & Boyce, 2009)

58	Cytomodulin-functionalized porous PLGA particulate scaffolds respond better to cell migration, actin production and wound healing in rodent model.	Anupama Mittal	Journal of Tissue Engineering and Regenerative Medicine	2014	(Mittal, Kumar, Parsad, & Kumar, 2014)
59	Collagen hydrogels strengthened by biodegradable meshes are a basis for dermo-epidermal skin grafts intended to reconstitute human skin in a one-step surgical intervention	Fabienne Hartmann-Fritsch	Journal of Tissue Engineering and Regenerative Medicine	2016	(Hartmann-Fritsch et al., 2016)
60	Human urine-derived stem cells in combination with polycaprolactone/gelatin nanofibrous membranes enhance wound healing by promoting angiogenesis	Yinxin Fu	Journal of Translational Medicine	2014	(Y. Fu, Guan, Guo, Guo, Niu, et al., 2014)
61	In vivo biocompatibility and vascularization of biodegradable porous polyurethane scaffolds for tissue engineering	M.W. Laschke	Acta Biomaterialia	2009	(Laschke et al., 2009)
62	Electrospun Composite Mats of Poly[(D,L-lactide)-co-glycolide] and Collagen with High Porosity as Potential Scaffolds for Skin Tissue Engineering	Ye Yang	Macromolecular Materials and Engineering	2009	(Y. Yang, Zhu, Cui, Li, & Jin, 2009)

63	In vitro and in vivo evaluation of electrospun nanofibers of PCL, chitosan and gelatin: A comparative study	S.R. gomes	Materials Science and Engineering: C	2015	(Gomes et al., 2015)
64	Thermosensitive Hydrogel PEG-PLGA-PEG Enhances Engraftment of Muscle-derived Stem Cells and Promotes Healing in Diabetic Wound	Pui Yan Lee	Molecular Therapy	2007	(P. Y. Lee, Cobain, Huard, & Huang, 2007)
65	Effect of Electrospun Non-Woven Mats of Dibutyryl Chitin/Poly(Lactic Acid) Blends on Wound Healing in Hairless Mice	Seon Il Jang	Molecules	2012	(Jang et al., 2012)
66	Evaluation of emulsion electrospun polycaprolactone/hyaluronan/epidermal growth factor nanofibrous scaffolds for wound healing	Zhenbei Wang	Nanotechnology in Biomaterials	2016	(Z. Wang et al., 2016)
67	The use of Adipose Tissue-Derived Stem Cells within a Dermal Substitute Improves Skin Regeneration by Increasing Neoangiogenesis and collagen Synthesis	Manuel Meruane	Plastic Reconstructive Surgery	2012	(Meruane, Rojas, & Marcelain, 2012)

68	Study In Vivo Intraocular Biocompatibilty of In Situ Gelation Hydrogels: Poly(2-Ethyl Oxazoline)-Block-Poly(E-Caprolactone)-Block-Poly(2-Ethyl Oxazoline) Copolymer, Matrigel and Pluronic F127	Yih-Shiou Hwang	PLOS One	2013	(Y.-S. Hwang et al., 2013)
69	Tissue Engineered Skin Substitutes Created by Laser-Assisted Bioprinting Form Skin-Like Structures in the Dorsal Skin Fold	Stefanie Michael	PLOS One	2012	(Michael et al., 2013)
70	Synthesis and characterization of model extracellular matrix that induces partial regeneration of adult mammalian skin	I V Yannas	PNAS	2089	(Yannas, Lee, Orgill, Skrabut, & Murphy, 1989)
71	Transdermal photopolymerization of minimally invasive implantation	J Elisseeff	PNAS	1999	(Elisseeff et al., 1999)
72	Nanofibrous rhPDGF-eluting PLGA-collagen hybrid scaffolds enhance healing of diabetic wounds	Chen-Hung Lee	RCS Advances	2016	(C.-H. Lee et al., 2016)
73	Fabrication of a triiodothyronine incorporated nanofibrous biomaterial: its implications on wound healing	Aishwarya Satish	RCS Advances	2015	(Satish & Korrapati , 2015)

74	Electrospun polycaprolactone membranes incorporated with ZnO nanoparticles as skin substitutes with enhanced fibroblast proliferation and wound healing	Robin Augustine	RSC Advances	2014	(Augustine et al., 2014)
75	Integrated poly-D,L-lactide-co-glycolide/silver nanocomposite: synthesis, characterization and wound healing potential in Wistar Albino rats.	Renu Sankar	RSC Advances	2016	(Sankar, Shivashangari, & Ravikumar, 2016)
76	Ibuprofen loaded PLA nanofibrous scaffolds increase proliferation of human skin cells in vitro and promote healing of full thickness incision wounds in vivo	M. Mohiti-Asli	Society For Biomaterials	2017	(Mohiti-Asli et al., 2017)
77	Vitalization of Porous Polyethylene (Medpor) with Chondrocytes Promotes Early Implant Vascularization and Incorporation into the Host Tissue	Susanne Ehrmantraut	Tissue Engineering Part A	2012	(Ehrmantraut et al., 2012)
78	Biocompatible Nanofiber Matrices for the Engineering of a Dermal Substitute for Skin Regeneration	J. Venugopal	Tissue Engineering	2005	(Venugopal & Ramakrishna, 2005)

79	A Rapid Fabricated Living Dermal Equivalent for Skin Tissue Engineering: An *In Vivo* Evaluation in an Acute Wound Model	Michael Ananta	Tissue Engineering Part A	2011	(Michael Ananta et al., 2011)
80	Vascularization of the Dermal Support Enhances Wound Re-Epithelialization by In Situ Delivery of Epidermal Keratinocytes	Liana M. Lugo	Tissue Engineering Part A	2010	(Lugo, Lei, & Andreadis, 2010)
81	Effect of artificial dermal substitute, cultured keratinocytes and split thickness skin graft on wound contraction	Michael J. Reid	Wound Repair and Regeneration	2007	(Reid, Currie, James, & Sharpe, 2007)
82	Androgen actions in mouse wound healing: Minimal in vivo effects of local antiandrogen delivery	Yiwei Wang	Wound Repair and Regeneration	2016	(Yiwei Wang et al., 2016)
83	Histological evaluation of skin reconstruction using artificial dermis	Matsui R.	Biomaterials	1996	(Matsui et al., 1996)

Table 7					
Papers With Polyester Collagen Scaffolds Used in This Analysis					
1	Nanofibrous rhPDGF-eluting PLGA-collagen hybrid scaffolds enhance healing of	Chen-Hung Lee	RCS Advances	2016	(C.-H. Lee et al., 2016)

	diabetic wounds				
2	Rapid creation of skin substitutes from human skin cells and biomimetic nanofibers for acute full-thickness wound repair	Sayed Babak Mahjour	Burns	2015	(Mahjour et al., 2015)
3	Effects of nanofiber/Stem cell composite on wound healing in Acute Full-Thickness Skin Wounds	Kun Ma	Tissue Engineering Part A	2011	(Ma et al., 2011)
4	The roles of knitted mesh-reinforced collagen-chitosan hybrid scaffold in the one-step repair of full thickness skin defects in rats	Wang Xingang	Acta Biomaterialia	2013	(Xingang Wang et al., 2013)
5	PLLA-collagen and PLLA-Gelatin Hybrid scaffolds with funnel-like porous structure for skin tissue engineering	Hongxu Lu	Science and Technology of Advanced Materials	2012	(Lu et al., 2012)
6	Augmentation of diabetic wound healing and enhancement of collagen content using nanofibrous glucophage-loaded collagen/PLGA scaffold membranes	Cheng-Hung Lee	Journal of Colloid and Interface Science	2015	(C.-H. Lee et al., 2015)

7	Mitigation of hypertrophic scar contraction via an elastomeric biodegradable scaffold	Elizabeth R. Lorden	Biomaterials	2015	(Lorden et al., 2015)
8	In vitro and in vivo evaluation of the wound healing capability of electrospun gelatin/PLLCL nanofibers	Guorui Jin	Bioactive and Compatible Polymers	2014	(Jin, Li, Prabhakaran, Tian, & Ramakrishna, 2014)
9	An elctrospun scaffold with anti-androgen receptor compound for accelerating wound healing	Cassandra Chong	Burns & Trauma	2013	(Chong, Wang, Maitz, Simanainen, & Li, 2013)
10	Functionalisation and surface modification of electrospun polylactic acid scaffold for tissue engineering	Elham Hoveizi	Cell Biology International	2014	(Hoveizi, Nabiuni, Parivar, Rajabi-Zeleti, & Tavakol, 2014)
11	A new, bioactive antibacterial-eluting, composite graft for infection-free wound healing.	Anupama Mittal	Wound Repair and Regeneration	2014	(Mittal & Kumar, 2014)

12	Engineered human sk in fabricated using ele ctrospun collagen-PCL blends: morphogenesis and mechanical properties.	Heather Powell	Wound Repair and Regeneration	2015	(Powe ll & Boyce, 2009)
13	An Investigation Study of Gelatin release from semi-interpenetrating polymeric network hydrogel patch for excision wound healing on Wistar rat model.	Maneesh Jaiswal	Journal of Applied Polymer Science	2014	(Jaisw al, Gupta, Dinda, & Koul, 2015)
14	Microporous Dermal-Like Electrospun Scaffolds Promote Accelerated Skin Regeneration	Paul Bonvallet	Tissue Engineering: Part A	2014	(Bonv allet et al., 2014; "Micro porous Derma l-Like Electro spun Scaffol ds Promo te Accele rated Skin Regen eration ," 2014)

15	The healing effect of unrestricted somatic stem cells loaded in collagen- modified nanofibrous PHBV scaffold on full-thickness skin defects	Saeed Heidari Keshel	Artificial Cells, Nanomedicine and Biotechnology	2014	(Keshel et al., 2014)
16	In vitro and in vivo evaluation of electrospun nanofibers of PCL, chitosan and gelatin: A comparative study	S.R. Gomes	Materials Science and Engineering: C	2015	(Gomes et al., 2015)
17	Multilayer Cell-Seeded Polymer Nanofiber Constructs for Soft-Tissue Reconstruction	Daniel Barker	JAMA Otolaryngology Head & Neck Surgery	2013	(Barker et al., 2013)
18	Small-diameter biodegradable scaffolds for functional vascular tissue engineering in the mouse model	Jason Roh	Biomaterials	2008	(Roh et al., 2008)
19	Human single-donor composite skin substitutes based on collagen and polycaprolactone copolymer	Niann-Tzyy Dai	Biochemical and Biophysical Research Communications	2009	(Niann-Tzyy Dai et al., 2009)
20	Biocompatible Nanofiber Matrices for the Engineering of a Dermal Substitute for Skin Regeneration	J. Venugopal	Tissue Engineering	2005	(Venugopal & Ramakrishna, 2005)

21	Improving the Cell Distribution in Collagen-coated Poly-Caprolactone Knittings	Weilum Sun	Tissue Engineering Part C: Methods	2012	(Sun et al., 2012)
22	A Poly(Lactic Acid-Co-Caprolactone)-Collagen Hybrid for Tissue Engineering Applications	M. Ananta	Tissue Engineering Part A	2008	(M. Ananta et al., 2008)
23	The three-dimensional vascularization of growth factor-releasing hybrid scaffold of poly (E-caprolactone)/collagen fibers and hyaluronic acid hydrogel	Ekaputra AK	Biomaterials	2011	(Andrew K. Ekaputra, Prestwich, Cool, & Hutmacher, 2011)
24	Collagen surface modified poly (E-caprolactone) scaffolds with improved hydrophilicity and cell adhesion properties	Ines Sousa	Materials Letters	2014	(Sousa, Mendes, Pereira, & Bártolo, 2014)
25	A novel poly (L-lactide-co-e-caprolactone) Collagen hybrid construct for application in tissue engineering	M. Ananta	TERMIS-EU	2007	(M. Ananta, Hilborn, Aibibu, Brown, & Muder a, 2007)

26	Spatial Arrangement of Polycaprolactone/Collagen Nanofiber Scaffolds Regulates the Wound Healing Related Behaviors of Human Adipose Stromal Cells	Fu X.	Tissue Engineering Part A	2011	(X. Fu & Wang, 2011)
27	In vivo biocompatibility of collagen poly(hydroxyethylmethacrylate) hydrogels	Jeyanthi R.	Biomaterials	1990	(Jeyanthi & Panduranga Rao, 1990)
28	In vivo promoted growth of mice hair follicles by the controlled release of growth factors	Ozeki M	Biomaterials	2003	(Ozeki & Tabata, 2003)
29	Initial experience with a composite autologous skin substitute	Robert L. Sheridan	Burns	2001	(Sheridan et al., 2001)
30	Markers to Evaluate the Quality and Self-Renewing Potential of Engineered Human Skin Substitutes In Vitro and after Transplantation	Pontiggia L.	Journal of Investigative Dermatology	2009	(Pontiggia et al., 2009)
31	Effect of Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) Nanofiber Matrices Cocultured With Hair Follicular Epithelial and Dermal cells for Biological Wound Dressing	Insook Han	Artificial Organs	2007	(Han et al., 2007)

32	Electrospun PLGA/collagen nanofibrous membrane as early-stage wound dressing	Shih-Jung Liu	Journal of Membrane Science	2010	(S.-J. Liu et al., 2010)
33	Collagen hydrogels strengthened by biodegradable meshes are a basis for dermo-epidermal skin grafts intended to reconstitute human skin in a one-step surgical intervention	Fabienne Hartmann-Fritsch	Journal of Tissue Engineering and Regenerative Medicine	2012	(Hartmann-Fritsch et al., 2016)
34	Bilaminar Device of Poly(Lactic-co-Glycolic Acid)/Collagen Cultured With Adipose-Derived Stem Cells for Dermal Regeneration	Juliana Domingues	Artificial Organs	2016	(Domingues et al., 2016)
35	Electrospun Composite Mats of Poly[(D,L-lactide)-co-glycolide] and Collagen with High Porosity as Potential Scaffolds for Skin Tissue Engineering	Ye Yang	Macromolecular Materials and Engineering	2009	(Y. Yang et al., 2009)
36	PLGA/gelatin hybrid nanofibrous scaffolds encapsulating EGF for skin regeneration	Mohammad Norouzi	Journal of Biomedical Materials Research Part A	2014	(Norouzi, Shabani, Ahvaz, & Soleimani, 2015)
37	Polycaprolactone-based fused deposition modeled mesh for delivery of antibacterial agents to	Erin Yiling Teo	Biomaterials	2011	(Teo et al., 2011)

	infected wounds				
38	Novel biodegradable sandwich-structured nanofibrous drug-eluting membranes for repair of infected wounds: an in vitro and in vivo study	Dave Wei-Chih Chen	International Journal of Nanomedicine	2012	(D. W.-C. Chen et al., 2012)
39	Fabrication and characterization of poly(l-lactide-co-glycolide) knitted mesh-reinforced collagen–chitosan hybrid scaffolds for dermal tissue engineering	Xingang Wang	Journal of Mechanical Behaviour of Biomedical Materials	2012	(Xingang Wang et al., 2012)
40	In vivo conjunctival reconstruction using modified PLGA grafts for decreased scar formation and contraction	Sang Young Lee	Biomaterials	2003	(S. Y. Lee et al., 2003)
41	Bioburden-responsive antimicrobial PLGA ultrafine fibers for wound healing	Somiraa Said	European Journal of Pharmaceutics and Biopharmaceutics	2012	(Said et al., 2012)

42	*In Vitro* and *In Vivo* Behaviors of the Three-layered Nanocarbonated Hydroxyapatite/Collagen/PLGA Composite	Susan Liao	Journal of Bioactive and Compatible Polymers	2010	(You et al., 2013)
43	Three types of dermal grafts in rats: the importance of mechanical property and structural design	Chuangang You	Biomedical Engineering Online	2013	(You et al., 2013)
44	Multilayer scaffold of electrospun PLA–PCL–collagen nanofibers as a dural substitute	Yu-fei Wang	Journal of Biomedical Materials Research Part B Applied Biomaterials	2013	(Yu-fei Wang, Guo, & Ying, 2013)
45	Fabrication of Novel Scaffolds Containing Collagen-I/Polylactic Acid/Nanohydroxyapatite via Co-electrospinning Methods	Zang Jun-ting	Chemical Research In Chinese University	2010	("Fabrication of Novel Scaffolds Containing Collagen-I/Polylactic Acid/Nanohydroxyapatite via Co-electrospinni

					ng Methods," n.d.)
46	Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds.	Zhang Y.	Journal of Biomedical Material Research B Applied Biomaterials	2005	(Y. Zhang, Ouyang, Lim, Ramakrishna, & Huang, 2005)
47	Co-electrospun poly(lactide-*co*-glycolide), gelatin, and elastin blends for tissue engineering scaffolds	Mengyan Li	Journal of Biomedical Materials Research	2006	(M. Li et al., 2006)
48	Synthesis and characterization of PLGA/collagen composite scaffolds as skin substitute produced by electrospinning through two different approaches	Ali Rexa Sadeghi-avalshahr	Journal of Materials Science: Materials in Medicine	2017	(Sadeghi-avalshahr, Khorsand-Ghayeni, Nokhasteh, Molavi, & Naderi-Meshkin, 2017)

49	Surface modification of nanofibrous polycaprolactone/gelatin composite scaffold by collagen type I grafting for skin tissue engineering	Sneh Gautam	Materials Science and Engineering:C	2014	(Gautam, Chou, Dinda, Potdar, & Mishra, 2014)

Table 8

Papers with Collagen Scaffolds Used to Test Precision.

	Paper Name	First Author	Journal	Year	Reference
1	The Use of a Pure Native Collagen Dressing for Wound Bed Preparation Prior to Use of a Living Bi-layered Skin Substitute	Naz Wahab	Journal of the American College of Clinical Wound Specialists	2014	(Wahab, Roman, Chakravarthy, & Luttrell, 2015)
2	The usefulness of the collagen and elastin sponge derived from salmon as an artificial dermis and scaffold for tissue engineering.	Matsumoto Y	Biomedical Research International	2011	(Matsumoto et al., 2011)
3	Cultured Human Keratinocytes on Type I collagen Menbranes to Reconstitute the Epidermis	Raymund Horch	Tissue Engineering	2000	(Horch, Debus, Wagner, & Stark, 2000)
4	Acellular Human Dermis Promotes Cultured Keratinocyte Engraftment	Hans Rennekampff	Journal of Burn Care & Rehabilitation	1997	(Rennekampff, Kiessig, Griffey, Greenleaf, & Hansbrough, 1997)
5	Closure of Abdominal Wall Defects Using Acellular Dermal Matrix	Gary An	The Journal of Trauma Injury, Infection, and Critical Care	2004	(An, Walter, & Nagy, 2004)

6	Role of wound healing myofibroblasts on re-epithelialization of human skin.	Veronique Moulin	Burns	2000	(Moulin, Auger, Garrel, & Germain, 2000)
7	Keratinocytes and Fibroblasts in a Human Skin Equivalent Model Enhance Melanocyte Survival and Melanin Synthesis After Ultraviolet Irradiation	Michael Archambault	Journal of Investigative Dermatology	1995	(Archambault, Yaar, & Gilchrest, 1995)
8	Skin Wound Closure in athymic mice with cultured human cells, biopolymers, and growth factors	Steven Boyce	Biomaterials	1991	(S. T. Boyce et al., 1991)
9	Surface Electrical Capacitance as a noninvasive Index of Epidermal Barrier in Cultured Skin Substitutes in Athymic Mice	Steven Boyce	The Society for Investigative Dermatology	1996	(S. T. Boyce et al., 1996)
10	Microstructure, rheological and wound healing properties of collagen-based gel from cuttlefish skin	Mourad Jridi	International Journal of Biological Macromolecules	2015	(Jridi et al., 2015)

Table 9

Papers with Polyester Scaffolds Used to Test Precision.

	Paper Name	First Author	Journal	Year	Reference
1	Aloe vera incorporated biomimetic nanofibrous scaffold: a regenerative approach for skin tissue engineering	S. Suganya	Iranian Polymer Journal	2014	(Suganya et al., 2014)
2	*In vivo* optimization of a living dermal substitute employing cultured human fibroblasts on a biodegradable polyglycolic acid or polyglactin mesh	Matthew Cooper	Biomaterials	1991	(Cooper et al., 1991)
3	Combined effects of PLGA and vascular endothelial growth factor promote the healing of non-diabetic and diabetic wounds	Kiran Kumar Chereddy	Nanomedicine: Nanotechnology Biology and Medicine	2015	(Chereddy et al., 2015)
4	Combined effects of PLGA and curcumin on wound healing activigty	Kiran Kumar Chereddy	Journal of Controlled release	2013	(Chereddy et al., 2013)
5	Fabrication and structure analysis of poly(lactide-co-glycolic acid)/silk fibroin hybrid scaffold for wound dressing	Sheida Shahverdi	International Journal of Biological Macromolecules	2014	(Shahverdi et al., 2014)

	applications				
6	Enhancement of Diabetic Wound Repair Using Biodegradable Nanofibrous Metformin-Eluting Membranes: in Vitro and in Vivo	Cheng-Huang Lee	ACS Applied Materials & Interfaces	2014	(C.-H. Lee et al., 2014)
7	Heparin-Conjugated Poly(lactic-co-glycolic acid) nanospheres enhance large-wound healing by delivering growth factors in platelet-rich plasma	Wan-Geun La	Artificial Organs	2014	(La & Yang, 2015)
8	Fish collagen-based scaffold containing PLGA microspheres for controlled growth factor delivery in skin tissue engineering	Huan Cao	Colloids and Surfaces B: Biointerfaces	2015	(Cao et al., 2015)
9	Controlled release of bioactive TGF-B from microspheres embedded within biodegradable hydrogels	Alicia DeFail	Biomaterials	2006	(DeFail, Chu, Izzo, & Marra, 2006)

10	The influence of pancrease-derived stem cells on scaffold based skin regeneration.	H. Salem	Biomaterials	2009	(Salem et al., 2009)

Table 10

Papers with Hybrid Scaffolds Used to Test Precision.

	Paper Name	**First Author**	**Journal**	**Year**	**Reference**
1	Efficient cutaneous wound healing using bixin-loaded PCL nanofibers in daibetic mice	Pinzon-Garcia AD	Journal of Biomedical Materials Research Part B: Applied Biomaterials	2016	(Pinzón-García et al., 2016)
2	Fish collagen-based scaffold containing PLGA microspheres for controlled growth factor delivery in skin tissue engineering	Huan Cao	Colloids and Surfaces B: Biointerfaces	2015	(Cao et al., 2015)
3	Experimental study on gelatin/polycaprolactam composite nanofiber scaffold in wound healing	Long JH	Journal	2006	(Long, Tan, Jiang, & Zhang, 2008)
4	Biocompatibility Evaluation of Electrospun Collagen, Gelatin, Polycaprolactone and their Composite	Anuradha Elamparithi	Indian Veterinary Journal	2016	(Elamparithi, Ravi, Balachandran, Rao, & Paul, 2016)

	Matrices in Rattus Norvegicus				
5	Three Dimensional Tissue Engineering Scaffolds And Growth Factor Delivery System Comprised Of Electrospun Hybrid Mesh Of Poly (Caprolactone)/Collagen Fibers And Hyaluronic Acid Hydrogel.	AK Ekaputra	*Tissue Engineering and Regenerative Medicine International Society Asia Pacific Meeting 2011*	2011	(A. K. Ekaputra, Prestwich, Cool, & Hutmacher, n.d.)
6	Engineered Human Skin Fabricated using Electrospun Collagen-PCL Blends	Heather Powell	Wound Repair and Regeneration	2015	(Powell & Boyce, 2009)
7	In vitro and in vivo evaluation of novel implantable collagen–chitosan–soybean phosphatidylcholine composite film for the sustained delivery of mitomycin C	Zhenqing Hou	Drug Development Research	2009	(Hou et al., 2009)
8	Vascularization of LBL structured nanofibrous matrices with endothelial cells for tissue regeneration	Lei Cui	RSC Advances	2017	(Cui et al., 2017)

9	A novel hydrogel-collagen composite improves functionality of an injectable extracellular matrix	R. Hartwell	Acta Biomaterialia	2011	(Hartwell et al., 2011)
10	Enhancement of Diabetic Wound Repair Using Biodegradable Nanofibrous Metformin-Eluting Membranes: in Vitro and in Vivo	Cheng-Huang Lee	ACS Applied Materials & Interfaces	2014	(C.-H. Lee et al., 2014)

Table 11

ANOVA results of healing rates across scaffold classes.

	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Scaffold Type	2	1.70943	0.85472	18.1677	<.0001
Error	778	36.6017	0.04705		
C. Total	780	38.3112			
Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Hybrid	164	0.17142	0.01694	0.13817	0.20466
Natural	334	0.17111	0.01187	0.14781	0.19441
Synthetic	283	0.26854	0.01289	0.24323	0.29385

Rsquare	0.04462
Adj Rsquare	0.04216
Root Mean Square Error	0.2169
Mean of Response	0.20648
Observations (or Sum Wgts)	781

Table 12

ANOVA Results of Healing Rates Across Fabrication Methods

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
ECM Source	7	2.51661	0.35952	7.7639	<.0001
Error	773	35.7946	0.04631		
C. Total	780	38.3112			

Rsquare	0.06569
Adj Rsquare	0.05723
Root Mean Square Error	0.21519
Mean of Response	0.20648
Observations (or Sum Wgts)	781

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Cell Line	3	0.52083	0.12424	0.2769	0.7647
decellularized	19	0.12144	0.04937	0.0245	0.2183
Decellularized	286	0.17922	0.01272	0.1542	0.2042
Electrospinning	402	0.2316	0.01073	0.2105	0.2527
Fused deposition Modeling	12	0.22902	0.06212	0.1071	0.351
Pressed	2	0.99844	0.15216	0.6997	1.2971
Purchased	48	0.16697	0.03106	0.106	0.2279
Sigma Aldrich	9	0.02976	0.07173	-0.111	0.1706

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